



## **The risk from SARS-CoV-2 to hazel dormice (*Muscardinus avellanarius*) in the United Kingdom and mitigation options relevant to conservation field workers**

### **Introduction**

SARS-CoV-2 is the name given to the newly evolved coronavirus which at the time of writing is responsible for the Covid-19 global pandemic in humans (Gorbalenya et al., 2020). SARS-CoV-2 belongs to the *Betacoronavirus* genus within the *Coronaviridae* family (de Groot et al., 2012; Masters, 2006). Coronaviruses are enveloped RNA viruses, have the largest genomes among all RNA viruses and are capable of infecting avian and mammalian species including humans and causing a variety of diseases (de Groot et al., 2012; Masters, 2006). For example, SARS-CoV-2 is a close relative of the coronaviruses MERS-CoV and SARS-CoV responsible for causing outbreaks of Middle East Respiratory Syndrome and Severe Acute Respiratory Syndrome respectively in humans in recent years. Both viruses are considered to have originated from animal reservoirs (Gorbalenya et al., 2020; Lu et al., 2020; Wassenaar & Zou, 2020). Reports suggest that SARS-CoV-2 originated from a free-living wild animal reservoir as is thought to be true for 60-70% of emerging diseases (Jones et al., 2008; Wang & Crameri, 2014). Although some coronaviruses are host specific, others appear capable of infecting multiple host species (Drexler et al., 2020). SARS-CoV-2 is likely to infect and replicate in numerous non-human mammalian host species as well as humans. Specifically, there is growing evidence that SARS-CoV-2 may be transmitted as a zoonosis from humans to animals.

The common, or hazel, dormouse (*Muscardinus avellanarius*) was included in Natural England's (formally English Nature's) Species Recovery Programme in 1992 (Mitchell-Jones & White, 2009) and has been designated as a UK Biodiversity Action Plan Priority species since 1997 due to the marked declines in numbers across the UK and the potential of climate change to substantially impact the survival of remaining populations. Since 1992, captive hazel dormice have been released at specially selected sites across England, aiming to increase numbers of remaining populations or to extend the range of the species back to historic levels (Mitchell-Jones & White, 2009; White & Hughes, 2019). As part of post-release monitoring activities dormouse nest boxes and footprint tunnels are established at reintroduction sites (Chanin & Gubert, 2011) and checked monthly between March and October each year. Fieldworkers therefore come into direct contact with hazel dormice through the handling of nest boxes, footprint tunnels and live hazel dormice throughout the monitoring season.

The purpose of this paper is to justify SARS-CoV-2 as a hazard to free-living hazel dormice, carry out a disease risk assessment, describe disease risk management options and discuss these in the context of the current Covid-19 pandemic.

## **Methods**

In this report we used the Sainsbury and Vaughan-Higgins' (2012) method of disease risk analysis (DRA) as developed from previous qualitative DRA methods for wildlife (Davidson and Nettles, 1992; Leighton, 2002) and using the foundation provided by the World Organization for Animal Health (Murray et al., 2004) and modified by Bobadilla-Suarez et al. (2017) and Rideout et al. (2017) to assess the magnitude and probability of disease occurring and propose methods to mitigate the risk from disease associated with carrying out conservation activities for free-living hazel dormice in the UK. Disease risk assessment was carried out according to the method described by the World Organization for Animal Health (Murray et al 2004). In addition, we carried out an exposure assessment for SARS-CoV-2 exposure in humans using the principles described by Murray et al (2004). We described the biological pathways that might permit hazel dormice and sympatric species at the destination to be exposed and infected with SARS-CoV-2 and the probability of this occurrence. We then described the processes required for SARS-CoV-2 to disseminate through hazel dormice and sympatric species populations and the probability of dissemination occurring. We assessed the likelihood and severity of biological, economic and environmental consequences associated with the establishment and spread of SARS-CoV-2. Using the method described in Murray *et al.* (2004), we combined the results of the exposure and consequence assessments to qualitatively assess the risk of disease associated with SARS-CoV-2 (negligible, very low, low, medium or high).

## **Results**

### **Hazard identification**

#### **Justification for SARS-CoV-2 as a hazard to Hazel dormice**

Here we justify SARS-CoV-2 as a hazard for free-living hazel dormice based on the likelihood of infection and disease in this species and the Family Gliridae, the severity of the disease and whether transmission can occur between dormice.

#### **Infection and disease associated with SARS-CoV-2-like coronaviruses in non-human mammals**

Studies have demonstrated that the ability of coronaviruses to replicate effectively within animal cells and to cause clinical disease may vary depending on the host species. For example evidence of infection with SARS-CoV has been detected in raccoon dogs (*Nyctereutes procyonoides*) and several bat species (*Rhinolophus spp.*) without reported clinical disease (Cheng et al., 2007; Guan et al., 2003; Li et al., 2005; Wassenaar & Zou, 2020). SARS-CoV-like viruses have however also been isolated from Himalayan palm civets (*Paradoxurus hermaphroditus*) shown experimentally to be susceptible to clinically detectable disease from two separate virus isolates (Guan et al., 2003; Z. Shi & Hu, 2008; Wu et al., 2005).

#### **Infection and disease associated with SARS-CoV-2 in non-human mammals**

A number of reports have highlighted the ability of SARS-CoV-2 to infect 12 non-human mammalian hosts: domestic cats (*Felis catus*), domestic dogs (*Canis familiaris*), house mice (*Mus musculus*), domestic pigs (*Sus scrofa domesticus*), domestic ferrets (*Mustela putorius furo*), fruit bats (*Rousettus aegyptiacus*), Syrian hamsters (*Mesocricetus auratus*), Malayan tigers (*Panthera tigris jacksoni*), Amur tigers (*Panthera tigris altaica*), African lions (*Panthera leo*), American mink (*Neovison vison*) and rhesus macaques (*Macaca mulatta*) (Balkema-Buschmann et al., 2020; Bao et al., 2020; Chan et al., 2020; Deng et al., 2020; Goumenou et al., 2020; J. Shi et al., 2020; Zhang et al., 2020). In eight of these mammalian species (domestic ferrets, Malayan tigers, African lions, domestic cats, fruit bats, Syrian hamsters, American mink and transgenic house mice) infection has been associated with disease (Balkema-Buschmann et al., 2020; Bao et al., 2020; Calle, 2020; Chan et al., 2020; J. Shi et al., 2020). Domestic pigs (*Sus scrofa domesticus*), domestic chickens (*Gallus gallus domesticus*) and domestic ducks (*Anas platyrhynchos*) are not thought to be susceptible to infection with SARS-CoV-2 (Balkema-Buschmann et al., 2020; Shi et al., 2020).

The virus has been shown to replicate effectively in the upper respiratory tract of Carnivora including ferrets (*Mustela putorius furo*) and cause clinical disease (Shi et al., 2020). Two ferrets in a study by Shi et al (2020) developed fever and loss of appetite 10 to 12 days after experimental inoculation with the virus. Post-mortem examination revealed evidence of lymphoplasmacytic perivascularitis and vasculitis, increased numbers of type II pneumocytes, macrophages, and neutrophils in the alveolar septa and alveolar lumen, and mild peribronchitis in the lungs, suggesting that ferrets are susceptible to the clinical disease associated with SARS-CoV-2. Balkema-Buschmann et al (2020) also demonstrated through experimental study that SARS-CoV-2 could replicate efficiently in ferrets and high viral RNA yields were detected in nasal washes from ferrets two to eight days post infection. In addition 100% (n=3) of non-inoculated ferrets which were kept in contact with experimentally infected ferrets also became infected and viral RNA was detected in nasal washing fluids from 12 days post-contact. SARS-CoV-2 reactive antibodies were detected from day 8 in the inoculated ferrets and in one in-contact ferret on day 21 (Balkema-Buschmann et al., 2020).

An outbreak of respiratory disease at two American mink farms in the Netherlands was thought to be associated with SARS-CoV-2 after clinically unwell animals at both farms tested positive for the virus (exact numbers not known) (ProMed International Society for Infectious Diseases, 2020a). This suggests that other members of the Mustelidae family may be susceptible to the disease and dissemination is through populations in close proximity may occur.

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

There is evidence to suggest that domestic cats (*Felis catus*) are susceptible to Covid-19 disease. Shi et al (2020) showed that the virus replicates effectively in cats and can transmit between them via respiratory droplets. Moreover, two juvenile cats in the same study which were experimentally

inoculated with SARS-CoV-2 were found to have severe lesions in the nasal and tracheal mucosal epithelia and lungs, highlighting their susceptibility to the disease (Shi et al., 2020). In a preliminary study in Wuhan, China, 102 serum samples were collected from domestic cats after the outbreak of SARS-CoV-2 in humans, and 14.7% (n=15) were positive for the receptor binding domain (RBD) of SARS-CoV-2 by indirect enzyme linked immunosorbent assay (ELISA), suggesting that SARS-CoV-2 infected the cat population in Wuhan during the outbreak (Zhang et al., 2020). There were also several case reports of owned domestic cats testing positive for SARS-CoV-2, for example a case in Belgium and a case in Hong Kong (News.gov.hk, 2020; ProMed International Society for Infectious Diseases, 2020b).

In the USA, a captive Malayan tiger (*Panthera tigris jacksoni*) from which duplicate nasal and oropharyngeal swabs were obtained tested positive on qPCR for SARS-CoV-2. The tiger had shown mild respiratory disease signs after contact with an infected keeper along with one other Malayan tiger, two Amur tigers (*Panthera tigris altaica*), and three African lions (*Panthera leo*) which were not confirmed to be positive for SARS-CoV-2 (Calle, 2020).

Since the Covid-19 outbreak was first reported, four domestic dogs have tested positive for SARS-CoV-2, and all had been in contact with an infected owner. None of the dogs showed signs of clinical disease, and although one dog died during the infection period, it was 17 years old and had multiple underlying diseases which were attributed as the cause of death rather than Covid-19 (Goumenou et al., 2020). Despite this evidence over 3500 dogs, cats and horses (*equus caballus*) showing respiratory disease screened for SARS-CoV-2 by IDEXX laboratories in South Korea in February and March 2020 tested negative (IDEXX, 2020). Given that there were 7,755 human patients with confirmed COVID-19 in Korea as of the 13<sup>th</sup> March 2020, this does suggest that whilst it remains possible for animals in contact with humans to become infected, occurrences are likely to be rare (Covid-19 National Emergency Response Center, 2020).

### **Infection and disease associated with SARS-CoV-2 in rodents**

Of rodent families closely related to the Gliridae, including Sciuridae (tree squirrels), Cricetidae (hamsters and voles) and Muridae (mice and rats), SARS-CoV-2 has been shown to infect and cause disease in Cricetidae and Muridae with evidence of Covid-19 in Syrian hamsters (*Mesocricetus auratus*) and transgenic mice (*Mus musculus*) within laboratory settings (Bao et al., 2020; Chan et al., 2020). Angiotensin 2 converting enzyme (ACE2) is a type I transmembrane metalloproteinase expressed in vascular endothelial cells, lungs, kidney, gastrointestinal tract and testes (Jiang et al., 2014; Ksiazek et al., 2003). Zhou et al., (2020) demonstrated that SARS-CoV-2 could utilise ACE2 to gain entry into human cells. In order to study the importance of ACE2 for SARS-CoV-2, hACE2 transgenic mice have been used as a disease model and compared to wild type mice. When intranasally inoculated with SARS-CoV-2, hACE2 transgenic mice show clinical signs of weight loss along with multiple histopathological changes including interstitial pneumonia. However, the same signs were not found in wild type mice inoculated with SARS-CoV-2, emphasising the importance of the ACE2 gene for disease in rodents (Bao et al., 2020). Syrian hamsters have been consistently experimentally infected with SARS-CoV-2 and have been found to show a range of clinical signs including rapid breathing and weight loss and histopathological changes from diffuse alveolar damage and apoptosis in the initial exudative phase to airway and intestinal involvement, spleen and lymphoid

atrophy and tissue repair in the later proliferative phase. Moreover, experimentally infected hamsters consistently infected naïve hamsters housed within the same cage, resulting in similar disease. It is likely that SARS-CoV-2 is able to utilise hamster ACE2 to enhance cellular entry (Chan et al., 2020). The ACE2 ortholog in hazel dormice has not, to the best of our knowledge, been fully sequenced and functionally investigated in the species. It remains plausible however that the encoded protein in the hazel dormouse may be a functional receptor for the spike glycoprotein of SARS-CoV-2 rendering the hazel dormouse susceptible to SARS-CoV-2 infection and Covid-19 disease. The susceptibility of mammals, to infection with SARS-CoV-2 and Covid-19 disease suggests that free-living wild mammals including rodents such as the hazel dormouse (family Gliridae) may be susceptible to infection and disease. To the best of our knowledge to date there have been no evidence of coronavirus infection in hazel dormice.

## **Disease Risk Assessment**

### **Human exposure assessment**

Within the United Kingdom (UK) cases of SARS-CoV-2 infection in humans number over 148,000 confirmed as of 27<sup>th</sup> April 2020 (World Health Organization, 2020b). Transmission of SARS-CoV-2 between humans occurs directly through aerosol droplets, spread by coughing or sneezing from an infected individual and through touching of contaminated surfaces (Kampf et al., 2020; Rothan & Byrareddy, 2020), as is the case with other coronaviruses (de Groot et al., 2012). Human infection is thought to occur through contact of viral particles with exposed mucous membranes including the eyes, nose and mouth (Lu et al., 2020; Zheng, 2020). Coronaviruses have been shown to persist on inanimate surfaces for up to nine days and, at low temperatures, persistence can be as long as 28 days (Ijaz et al., 1985; Kampf et al., 2020), although experimental evidence suggests that the survival on SARS-CoV-2 is likely to be 72 hours on stainless steel and plastic (van Doremalen et al., 2020). Viral RNA has been detected in nasal washes of ferrets inoculated with the virus, as well as in several upper respiratory tract structures of inoculated and exposed cats (Shi et al., 2020).

SARS-CoV-2 has also been detected in faeces of humans, a Malayan tiger and an African lion and is thought to be present in the faeces of bats (Calle, 2020; Holshue et al., 2020; Wassenaar & Zou, 2020) therefore faecal-oral transmission may also be possible, as for other closely related coronaviruses (Yeo et al., 2020). Rectal swabs taken from experimentally inoculated ferrets tested positive for viral RNA, though at lower levels than nasal washes. Infectious virus was not detected in any rectal swabs. In the same study, rectal swabs from experimentally inoculated beagles also tested positive for viral RNA (Shi et al., 2020).

The reproductive number  $R_0$  for SARS-CoV-2 is considered high with suggestions that in a naïve human population an average of 2-4 new infections may be generated from a single infectious human (Liu et al., 2020). The average incubation period is estimated to be between 2-14 days and it is not known to what extent shedding of the virus may occur within this period prior to the onset of clinical signs (Mizumoto et al., 2020; Yee et al., 2020). The availability of tests for SARS-CoV-2 for non-essential human workers in the UK remains low and knowledge as to whether an individual showing no or mild clinical signs is infected or not is likely to be unknown. Based on the current epidemiological

understanding of SARS-CoV-2 in humans there is a high likelihood of human exposure to SARS-CoV-2, the likelihood of infection after exposure is high and the likelihood of dissemination through the human population is high.

### **Dormouse exposure assessment**

Fieldworkers routinely check footprint tunnels and nest boxes at sites where hazel dormice have been released as part of conservation initiatives. Fieldworkers may be infected with SARS-CoV-2 and may expose dormice through aerosol droplets from the respiratory system if they in close contact with dormice. Dormice will also be exposed when footprint tunnels and nest boxes are handled and hazel dormice subsequently come into contact with virus particles whilst utilising tunnels and nest boxes. There is a low likelihood that hazel dormice will be exposed to SARS-CoV-2 from fieldworkers in this situation.

Fieldworkers handle dormice in order to carry out routine visual examinations and to obtain a body weight. Fieldworkers may be infected with SARS-CoV-2 and may sneeze or cough in the direct vicinity of the animal. There is a medium likelihood that hazel dormice will be exposed to SARS-CoV-2 in this situation.

Since hazel dormice share their environment with sympatric rodent species and both footprint tunnels and nest boxes may be entered by other rodents there is a low likelihood of other rodent species becoming exposed to SARS-CoV-2 through encounters with infected humans or hazel dormice. This exposure may occur directly through fomites or during encounters such as resource sharing or fighting.

SARS-CoV-2 has also been detected in faeces of humans (Holshue et al., 2020) and is thought to be present in the faeces of bats (Wassenaar & Zou, 2020). Rectal swabs taken from experimentally inoculated ferrets tested positive for viral RNA, though at lower levels than nasal washes and infectious virus was not detected in any rectal swabs. (Shi et al., 2020). Faecal-oral transmission therefore remains a further possible route of low likelihood through which hazel dormice may become exposed. Overall there is a medium likelihood of hazel dormice becoming exposed to SARS-CoV-2 during human fieldwork activities.

There is no evidence to suggest that if hazel dormice are exposed, they will become infected, but two other rodent species have been infected after experimental intranasal inoculation, and the lack of research in this area means the eventuality of hazel dormice becoming infected cannot be ruled out. There is a medium likelihood that hazel dormice will become infected with SARS-CoV-2 if exposed. The probability of the virus being disseminated amongst the hazel dormouse population is medium, since rodent to rodent transmission has been shown for Syrian hamsters. Animal to animal transmission has also been shown for domestic cats and ferrets.

### **Consequence assessment**

There is evidence that two other species within the Rodentia order became infected and developed clinical signs after experimental intranasal inoculation with SARS-CoV-2 (Bao et al., 2020, Chan et al.,

2020). Covid-19 disease has been shown to occur in one non-transgenic species of rodent infected with SARS-CoV-2 in the laboratory, the Syrian hamster. Clinical signs in infected Syrian hamsters were considerable but did not result in mortality (Chan et al., 2020). Wild-type house mice did not appear to be susceptible in a separate study (Bao et al., 2020), implying that susceptibility is likely to be variable among rodent species. The pathogenesis of SARS-CoV-2 in other non-laboratory rodent species and in particular free-living wild rodents is unclear although the literature so far suggests that severe disease and death is unlikely to occur after exposure. At this stage there is a low likelihood of severe disease and mortality in hazel dormice if they were to become infected and a very low likelihood of a disease outbreak in hazel dormice at the field site. There is a very low likelihood of economic consequences if an outbreak of Covid-19 disease were suspected in the hazel dormouse population, with a need for fieldworkers to wear full personal protective equipment (PPE) to increase population monitoring and disease surveillance activities including for collaborating parties to pay for testing of both human and animal subjects.

### **Risk estimation**

Based on the current understanding of SARS-CoV-2 there is a high likelihood of exposure, infection and dissemination of SARS-CoV-2 in the human population and a medium likelihood that hazel dormice will become exposed and infected through human fieldwork activities at conservation sites. There is a low likelihood that SARS-CoV-2 will disseminate through the hazel dormouse population. . There is a low likelihood of biological and economic consequences through severe disease and a disease outbreak in dormouse populations. The overall risk of SARS-CoV-2-associated disease in hazel dormice is estimated to be LOW.

### **Risk management**

#### **Risk evaluation**

Although the overall risk estimation is considered low this is greater than negligible therefore risk management measures need to be employed.

#### **Option evaluation**

The objective is to reduce the exposure of hazel dormice to SARS-CoV-2 from human fieldworkers.

To reduce the risk of exposure of the hazel dormouse population to SARS-CoV-2 careful consideration should be given as to the necessity of each monitoring visit to a conservation site. Current UK government guidance should be followed with respect to minimising travel, avoiding public transport and being in the company of a limited number of other personnel. Fieldworkers showing clinical signs of Covid-19 disease or who have been in contact with a person displaying symptoms within the last 14 days should not undertake fieldwork activities. All such persons should seek SARS-CoV-2 testing and if at all possible obtain a clear test result or self-isolate for a minimum of 14 days before commencing fieldwork.

Despite following these rules, symptom-based screening of humans to prevent disease transmission is likely to be ineffective at preventing transmission due to the risk of infected but asymptomatic hosts, and further measures should be implemented to stop viral spread (Hoehl et al., 2020). Currently the use of personal protective equipment (PPE) and good hygiene are considered to be the most effective measures against transmission of the virus (Yee et al., 2020). Personnel undertaking fieldwork activities should therefore adhere to strict biosecurity principles. It is recommended that a disposable overall is donned before entering the conservation site. Hand cleaning should be undertaken at regular intervals throughout fieldwork activities, either washed with soap and water for a minimum of 20 seconds (following the World Health Organisation (WHO) guidelines (World Health Organization, 2020a)), or cleaned by liberally using a hand sanitiser with at least 70% alcohol as an active ingredient, since this has been shown to be effective at killing SARS-CoVs in 30 seconds (Siddharta et al., 2017; World Health Organization, 2009). Hand cleaning should particularly be undertaken before entering a field site, after touching any monitoring equipment and if the fieldworker touches their face. Disposable gloves should be worn. The effectiveness of face coverings as a means of preventing exposure to SARS-CoV-2 is currently unclear however there appears to be some support for the wearing face coverings by potentially infected humans to prevent respiratory droplet spread of virus particles (del Rio & Malani, 2020). Given the possibility that a fieldworker could be infectious whilst asymptomatic it is recommended therefore that face coverings are worn to convey additional protection against introducing SARS-CoV-2 into the hazel dormouse population. Although medical grade face masks, made from a minimum of three layers of synthetic, non-woven materials with filtration layers between, have been recommended by WHO (World Health Organization 2020c), the risk of shortage of these masks means that they should be reserved for use in healthcare settings; it has been suggested that these masks are more important in situations where self-protection is the priority (Greenhalgh et al. 2020). When considering the use of protective equipment to reduce the risk of exposing others, including hazel dormice, to SARS-CoV-2, for example from an infected fieldworker, a cloth face covering should suffice (Cheng et al. 2020). Cloth face coverings have been recommended by the Centers for Disease Control (CDC) as a method of minimising transmission from infected individuals (Centers for Disease Control 2020), and are suggested to be an appropriate alternative to medical grade face masks in the contexts of reducing transmission (Greenhalgh et al. 2020). Any face covering should be worn tightly around the chin and top of the nose and hand cleaning should be undertaken before placing the mask (World Health Organization 2020c).

To reduce the risk of exposure of hazel dormice during fieldwork activities additional measures are recommended when inspecting footprint tunnels and checking nest boxes. It is recommended that handling of dormice is minimised and in particular the eyes, nose or mouth of dormice should not be touched but rather visually inspected. Likewise handling of footprint tunnels and nest boxes should be minimised. During routine cleaning activities including of tunnels, boxes and any other equipment careful consideration of disinfectants should be given. Disinfectants containing 0.1% sodium hypochlorite or 62-71% ethanol lead to effective inactivation of the SARS-CoV-2 (Kampf et al., 2020) however the safety of products containing these chemicals has not been evaluated for use on footprint tunnels and nest boxes utilised by hazel dormice. At present Safe4 is considered the disinfectant of choice because it is safe for animals in direct contact even when surfaces remain damp with the product. Safe4 is also biodegradable and considered safe for the environment. The efficacy of Safe4 against SARS-CoV-2 has been evaluated and this product is considered effective against the virus at a

dilution of 1:50 (<https://www.safe4disinfectant.com/news.php>, n.d.). Fieldworkers moving between footprint tunnels and nest boxes within a conservation site should ideally change gloves or use Sterilium hand sanitiser to clean gloves between handling different tunnels, boxes and dormice. To avoid transfer of SARS-CoV-2 via fomites personal items such as watches and mobile phones should not be touched whilst carrying out fieldwork activities. At the end of the fieldwork site visit all potentially contaminated items including disposable overalls, gloves and masks should be placed in a clinical waste bin bag secured with a cable tie and disposed of appropriately. Hands should once again be cleaned with soap and water for a minimum of 20 seconds or by using hand sanitiser.

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