Final Beaver Trapping and Health Screening Report

River Otter Beaver Trial

Report prepared for Devon Wildlife Trust

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Summary

- Full health screening of the original founder beavers did not demonstrate any evidence of significant zoonotic disease, including *Giardia* spp., *Salmonella* spp., *Campylobacter* spp., *Yersinia* spp., *Cryptosporidium parvum, Echinococcus multilocularis, Franciscella tularensis* and *Mycobacterium* spp.
- Exposure and seroconversion to *Leptospira* spp. was evident in one of the founder beavers and three others over the five-year trial. Subsequent testing showed waning of the antibody response with no clinical disease being evident suggesting these animals were not persistently infected.
- No evidence of significant clinical disease in the beavers on the River Otter during the 5 years with overall good body condition being maintained from one year to the next.

Contents

1. Introduction

1.1. Background to River Otter beavers

Two families of breeding beavers were reported on the River Otter, Devon, in February 2014. After a successful public campaign to see them remain in place, Natural England (NE) granted a 5-year licence to Devon Wildlife Trust (DWT) in 2015. The River Otter Beaver Trial (ROBT) is a scientifically monitored trial reintroduction of Eurasian beavers (*Castor fiber*). ROBT is due to conclude in March 2020 when final reports will be submitted to NE on the conclusion of 5 years of scientific monitoring of beavers living within this catchment. A significant part of the granting of the licence was that only beavers certified as healthy and fit for release by a qualified veterinary surgeon were to be released. Specifically, they must be confirmed as being free from the Taeniid *Echinococcus multilocularis* (*Em*). One of the main goals and objectives of the ROBT is to establish a healthy population of Eurasian beavers into a lowland English river catchment.

1.2. Background to beaver health screening

The importance of animal health in conservation programmes is increasingly recognised as the success of any reintroduction can be significantly affected by disease. Despite this, the implementation and development of pre-reintroduction (e.g. robust disease risk analysis) and post-release (e.g. wildlife surveillance programmes) veterinary health programmes tend to receive less investment compared to other project aspects (Jamieson & Lacy, 2012). The establishment of baseline species-specific health parameters and routine analyses of diagnostic samples allows informed decision-making and improvement in animal health and welfare (Jakob-Hoff *et al*., 2015). However, as stated in the IUCN Reintroduction Guidelines, 'the level of attention to disease and parasite issues around translocated organisms and their destination communities should be proportional to the potential risks and benefits identified in each translocation situation' (IUCN/SSC, 2013, p10). Therefore, level of post-release health monitoring should reflect the assessed level of risk. Given the high-profile nature of beaver reintroduction and the need to assess their success, some level of systematic post-release health surveillance would be recommended.

Goodman *et al.* (2012) describe health surveillance protocols for beavers reintroduced to Scotland as part of the official scientific trial, and based on IUCN guidelines for reintroductions (IUCN/SSC, 2013). Along with pre-release health checks these guidelines stress the importance of post-release monitoring as a significant component in evaluating any reintroduction process. As part of any responsible reintroduction programme or trial, pre- and post-release health assessments are essential to ensure health and welfare legislation is complied with. One important method of health assessment in any animal is to assess haematological and biochemical parameters (Milner *et al*., 2003) along with general parasitology and bacteriology assessment. This provides a means to evaluate both the level to which the released animals and their offspring are coping in their habitat and the suitability of a release location. Blood haematology and serum chemistry have been used widely for beaver health assessment in British beaver restoration projects and therefore provide further comparable data (Campbell-Palmer *et al.,* 2015a; Girling *et al*., 2015; Goodman *et al*., 2012).

Health screening prior to any beaver release has two primary functions. Firstly, to ensure that any individuals are screened to ensure they present no risk of transmitting non-native parasite and diseases of concern; and secondly to ensure they are healthy and capable of coping with the release process (Animal Welfare Act, 2006).

The range of pathogens that can be harboured by the Eurasian beaver has been previously reviewed along with pre-release health screening recommendations for beaver importation to Scotland (Goodman *et al.*, 2012; Campbell-Palmer & Rosell, 2013). Beavers can carry host-specific parasites not currently or normally present in Britain, though these are not known to infect or harm other species. These include the beaver beetle *Platypsyllus castoris*, a stomach nematode *Travassosius rufus*, and a specialised trematode or intestinal fluke *Stichorchis subtriquetrus*. These species have now all been recorded in wild beavers in Scotland (Campbell-Palmer *et al*., 2013; Goodman *et al*., 2012; Duff *et al*., 2013). These non-native, host-specific parasites are not of concern to human, livestock or other wildlife health, so no active management for these species is presumed to be required. Other parasites such as *Giardia* spp. and *Cryptosporidium* spp. are already present in British wildlife and domestic animals, therefore it is likely that beavers may also act as carriers. Like all other rodents, beavers may harbour common European rodent pathogens (Goodman *et al*., 2012). For any beavers of unknown origin, a non-native disease and parasite concern would be the presence of *Em*, rabies and tularaemia (*Francisella tularensis*). However, it should be noted that serious consideration should be given to ensuring that released animals should be selected from captive bred or British wild individuals to minimise the risk particularly of *Em* transmission.

Currently the most significant diseases and parasites associated with beaver reintroduction (i.e. those which are Notifiable under EU Animal Health legislation and/or are likely to result in significant disease to domestic animals and humans) and requiring further screening or assessment of risk during release and translocations according to DEFRA are considered to be *Em*, *Francisella tularensis*, Rabies, *Leptospira* spp., *Cryptosporidium* spp., *Mycobacterium bovis* (bovine tuberculosis), *Salmonella* and *Giardia* spp. From a health and biosecurity perspective, beavers are currently considered to present no greater risk to human, livestock, or other wildlife health than any other native mammal.

Em - is a zoonotic parasite of serious health concern, regarded as one of the most pathogenic parasitic zoonoses in the Northern hemisphere (North America, northern and central Eurasia) (Eckert *et al*., 2000; Vuitton *et al*., 2003). *Em* has been identified in Eurasian beavers from Switzerland, Austria, Germany and Serbia (Janovsky *et al*., 2002; Cronstedt-Fell *et al*., 2010; Cirović *et al*., 2012; Wimmershoff *et al*., 2012). Although it is established in fox (*Vulpes Vulpes*) populations in many countries across Central Europe, other European countries are presently deemed free of this parasite – including the UK, which employs strict measures to prevent entry, i.e. the Pet Travel Scheme (DEFRA, 2012). Diagnosis in intermediate (non-egg-shedding) hosts such as beavers has historically been via post-mortem examination. Campbell-Palmer *et al*. (2015b) found that laparoscopic examination when combined with ultrasound investigation for real-time diagnosis of *Em* in beavers will allow the direct rapid identification of any abdominal lesions. Additionally, submission of blood samples for immunoblotting can be undertaken to identify any early cases so raising sensitivity testing to 85% (Gottstein *et al*., 2014). Barlow *et al*. (2011) diagnosed *Em* in a captive beaver at post-mortem. This individual was held in an English captive collection but had been directly wild-caught and imported years previously from Bavaria, Germany. Sample screening across the Tay and Earn catchments, and ongoing post-mortem examination of beaver cadavers, have demonstrated no evidence of *Em* in free-living beavers in Scotland. The occurrence of positive individuals in directly imported beavers (n=2) has drawn significant attention to the potential risk posed by unscreened beavers, although the likely risk has been evaluated as negligible (Kosmider *et al*., 2013).

Franciscella tularensis – is the causal agent of tularemia. It is transmitted by blood sucking insects

predominantly and has been reported in *Castor canadensis*, Eurasian brown hares (*Lepus europaeus*) along with many other species of rodent (Yarto-Jaramillo, 2015). It is a serious plague-like zoonotic condition with a significant mortality rate. Although in North America, beavers have been reported to be a significant reservoir (Morner, 1992), the Eurasian beaver is only reported sporadically as a host (Schulze *et al*., 2016). Currently it is not present in the UK and would only be an issue in imported animals. A commercial PCR is available to check blood or tissue samples for evidence of the bacterium.

Rabies - The rabies virus has not been reported in Eurasian beavers but theoretically may affect any mammal. Screening of the live animal is not currently possible, so any imported beavers should be sourced from rabies-free areas or quarantined according to the current Rabies Importation Order 1974 (as amended).

Leptospira spp. - has been regularly reported in rodents and has been reported at a low level in beavers and associated with *Yersinia* spp. infections and mortalities in Eurasian beavers (Nolet *et al*., 1997; Marreros *et al.,* 2018). It is theoretically possible beavers could pose a potential source of *Leptospira* spp. to other animals post-release, but persistent carrier status has yet to be demonstrated and seropositivity levels are considered low and these combined with the ubiquitous nature of *Leptospira* spp. in the UK makes the risk of leptospirosis associated with Eurasian beaver reintroduction low.

Cryptosporidium parvum - infection in beavers has been reported in Poland (Bajer *et al*., 1997) and North America (Isaac-Renton *et al*., 1987). One kit born in Scotland tested positive for *Cryptosporidium* spp. oocysts on a faecal sample obtained at post-mortem (Goodman *et al*., 2012). No significant increase in the prevalence of *Cryptosporidium* oocysts were found in any of the watercourses within the Scottish beaver trial (SBT) site (44 km^2 area of land in total) or any incidences of human cases during the five years of monitoring (Mackie, 2014). This, combined with the ease of diagnosis, would suggest that the risk of introducing clinically significant levels of *C. parvum* to the environment with a beaver release is small.

Mycobacterium bovis - has never been reported in the Eurasian beaver but theoretically any mammal can become infected so there is a small but identifiable risk. *Mycobacterium microti*, which is part of the Mycobacterium tuberculosis complex, has been reported in other rodents in the UK including field voles (*Microtus agrestis*), bank voles (*Clethrionomys glareolus*), wood mice (*Apodemus sylvaticus*) and shrews (*Sorex araneus*) in northern England but again never in the Eurasian beaver (Cavanagh *et al*. 2002; McClure, 2012). Testing for mycobacteria can be difficult as culture, the gold standard, takes a minimum of 3 months on specialized media (Yarto-Jaramillo, 2015). More rapid testing using acid-fast staining of lung washes and or real time PCR may also be used with lung radiography to rule-out infection in wild beavers screened in Britain (Campbell-Palmer *et al*., 2015b; Campbell-Palmer *et al*., 2015c). As a serious human and domestic animal pathogen, but with no data of infection in *Castor* spp., *M. bovis* has to be considered a very low disease risk from a reintroduced Eurasian beaver.

Salmonella spp. - have also been reported from Eurasian beavers in Europe but not currently in wild beavers in Scotland (Romasov, 1992; Rosell *et al*., 2001; Goodman *et al*., 2012; Campbell-Palmer *et al*., 2015a). It can easily be screened via faecal culture.

Giardia spp. – is a common intestinal parasite of many mammals. In North America, the prevalence of *Giardia* infection in beavers is 7-16% (Erlandsen *et al*., 1990) and beavers are considered a potential health risk if inhabiting drinking water reservoirs (Wallis *et al*., 1996). No significant increase in the prevalence of *Giardia* spp. cysts were found in any of the watercourses within the trial site (44 km^2 area

of land in total) during the course of monitoring (Mackie, 2014).

Individual beavers will present varying screening requirements prior to release depending on their sourcing. To reduce risk of introducing non-native parasites and diseases, and/or potentially acting as a reservoir for those of concern, UK captive born or Scottish wild born beavers are favoured for release. Even within these, any individuals of unknown and/or unproven origin are recommended to be more thoroughly assessed for non-native parasites and diseases, particularly *Em* (e.g. older Scottish animals).

To assess suitability for release it would be recommended that any pre-release health assessment should be carried out with specialist veterinary support and refer to current published baseline parameters for the Eurasian beaver (Goodman *et al.*, 2012; Cross *et al*., 2012; Girling *et al*., 2015). These individual assessments should follow previous health screening protocols undertaken for DWT, Tayside and SBT. This includes a general health assessment based on; physical examination, full haematology and serum biochemistry, parasitology and bacteriology, and general serology. Any individuals testing positive to host specific parasites, and/or common wildlife disease already present in British wildlife should not be automatically excluded from any release, though their fitness upon release and likely welfare status assessed on a case by case basis. Any individuals testing positive for *Em*, tularaemia or Hantavirus should not be released, and the Animal and Plant health Agency (APHA) contacted.

1.2.1 Re-release of original individuals

The origin and health status of the original River Otter beavers were unknown and therefore of concern particularly if they carried non-native parasites or diseases. As a condition of re-release, APHA requested and funded screening specifically for *Em*. All additional screening of these animals was funded by DWT and followed procedures employed by RZSS for assessment of the Tayside population, Perthshire, also composed of unofficial releases of animals of unknown origin. This included notifiable diseases and those of concern especially by agricultural stakeholders e.g. tularaemia, bovine tuberculosis (*Mycobacterium bovis*). There was also a need to clarify that the North American species (*C. canadensis*) has not been released and that any trapped beavers were Eurasian. Genetic analysis was also undertaken to establish the degree of relatedness and genetic diversity of these animals to aid in informing any further animal releases. An additional consideration of the screening was to assess the body condition and draw some inference on the adaptability of the trapped beavers to survive in an English landscape after an absence of over 400 years.

1.2.2 Ongoing assessment of trial animals

Ongoing assessment of trial animals mainly involved an annual trapping period that varied in timing and length of effort but largely occurred between Jan-Mar, working 3-5 active territories for 1-2 weeks. The main aims of these infield assessments were to trap and tag new animals (kits born that breeding season or previously un-trapped individuals); weigh and assess body condition; check micro-chips and re-tag ear tags as required; collect samples for veterinary screening where possible and map population distribution and composition according to individuals trapped. There were no officially requested disease and parasite screening requirements, so opportunistic screening to provide further information on overall population health post-release was undertaken in line with best practice recommendations (IUCN/SSC, 2013).

1.2.3 Release of additional individuals

The licence issued by Natural England (Feb 2015) details the following conditions which are relevant to the release of additional animals:

- All beavers to be released must be the Eurasian beaver and have either been taken under licence from the River Otter or sourced from a legally obtained, captive population.
- The release of additional beavers not formerly living on the River Otter may only be undertaken with specific written permission from NE.
- Only beavers certified as healthy and fit for release by a qualified veterinary surgeon are to be released. Specifically, they must be confirmed as being free from the Taeniid *Em*.
- All beavers released must be marked with permanent identification chips and an individually identifiable ear tag. This includes any beavers caught subsequently during the project that are found not to have an identification chip.
- Information on sex, genetic profile and approximate age of each beaver released from captivity must be obtained and documented prior to release.
- Information on approximate age and sex must be obtained for all field caught animals.
- Any known deaths of beavers must be reported to NE. If the carcass is available, a post-mortem must be carried out by a suitably experienced veterinary surgeon and the report copied to NE.
- Any reports of beaver in adjacent catchment areas must be reported to NE and followed up by the licensee. If confirmed, all reasonable attempts must be made by the licensee to trap and identify the beaver(s). NE must be involved in the decision of what to do with any captured beavers.
- The release of beavers must be undertaken in accordance with best practice, e.g. using 'soft release' techniques.

1.3. Objectives of this report

The main objectives of this report are to describe:

a) Requirements and findings of the original, pre-release health screening to meet trial licence conditions

b) Ongoing trapping and general health surveillance findings

c) Health screening requirements and release of additional animals

d) Conclusions on welfare and adaptation to River Otter habitat

e) Proposals for future monitoring

2. Methods

2.1. Trapping procedures

Of the original beavers reported on the River Otter, five individuals (presumed to be living as two family units) were live trapped using Bavarian beaver traps by APHA staff, then housed for a few weeks in captive facilities at Derek Gow Consultancy, Devon.

Subsequently all trial animals were trapped using Bavarian beaver traps supplied by Derek Gow Consultancy, annually in short periods, generally from Sept-March, though trapping time and effort varied. Landowner permission was sought by DWT. Trap placement and relocation were undertaken by RCP, Ed Lagdon and Jake Chant. Animal handling, tagging and sample collection were conducted by RCP, with blood samples, when collected, taken by veterinary surgeons from RZSS and/or New City Vets, Honiton.

Trap placement criteria included:

- Landowner permission
- Minimal interference from public
- Evidence of fresh beaver field sign
- Not subjected to sudden water level fluctuation
- Evidence of new territory establishment

Traps where baited regularly and checked daily. Any trapped individuals were removed from traps and restrained by experienced personnel using specialized equipment. After processing, the beaver was released immediately at point of capture.

Figure 1. Typical live trap set up with camera trap monitoring.

2.2. Screening procedures and samples collection

2.2.1 Original animals

Veterinary

All beavers were anaesthetised using 4% isoflurane in 100% oxygen via a face mask, then intubated and maintained on 1.5-2% isoflurane in 100% oxygen.

A full physical examination was undertaken whilst anaesthetised, including:

- eyes – symmetry of head, eyes for ocular discharge
- ears – check for parasites
- nose – check for nasal discharge, abrasions
- teeth – check for malocclusion, signs of dental disease, abdominal wear, trap injuries
- integument (including tail and feet) – check for wounds, ectoparasites, dermatitis, condition of fur, covering of fat over the pelvic region, spine and tail was assessed
- tail – check for wounds, abrasions, thickness

Each beaver was assessed for scars or any signs of previous trauma, as well as for the presence of any external parasites. Palpation was performed of all the limb joints to ensure normal range of motion, along with an abdominal palpation for any organ enlargements or abnormal masses. Fur condition was assessed as lack of proper grooming may represent underlying health issues and poorer body condition. Weight was measured and body score assessed according to the standard rodent body scoring system. Each beaver was scanned for the presence of an identity microchip, and if not present beavers were microchipped in the inter-scapular region to allow future identification.

Sex was initially established through the examination of the colour and viscosity of the anal gland secretions (AGS). The sex of each individual was further confirmed through laparoscopic examination. Blood was taken aseptically from the ventral tail vein for diagnostic testing and an additional sample taken for genetic screening. Haematology and serum biochemistry were performed as a general assessment of each beaver's general state of health (SAC Consulting Veterinary Services, Scotland's Rural College). Further specific serological testing was performed as follows: European Leptospira serovars (pools 1-6) using the microscopic agglutination test (MAT) (APHA, Weybridge); EM by means of two different enzyme-linked immunosorbent assays (ELISAs) targeted against the EM 18 and EM 2 antigens, used for human EM diagnosis, as well as a recently developed immunoblot. A specific anti-beaver IgG conjugate was used for testing at University of Bern, Switzerland. Polymerase chain reaction (PCR) testing was also carried out for tularaemia on serum (National Veterinary Institute, Norway).

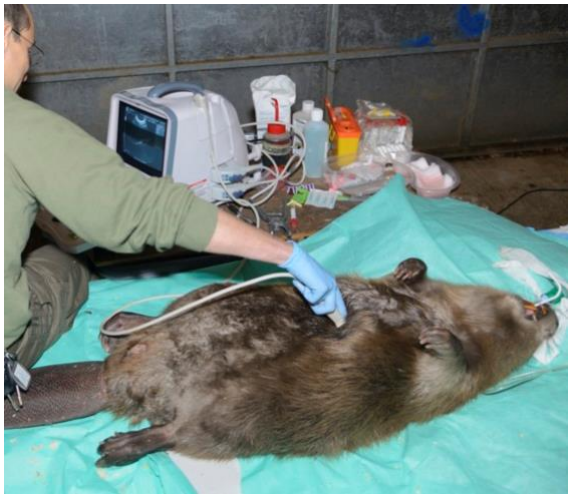

Figure 2a and b. Full health screening of the original beavers by RZSS, 2015.

Faeces were taken directly from the beaver's rectum, and rectal microbiology swabs were taken. Faecal samples underwent flotation with saturated salt solution for nematodes and sedimentation for trematodes, as well as microscopy for coccidia, *Cryptosporidium* spp., and *Giardia* spp. and acid-fast staining for *Mycobacterium avium* subsp. *paratuberculosis* (Johne's disease). Standard microbiological culture for bacterial enteric pathogens, including enriched media for *Salmonella* was performed (SAC Consulting Veterinary Services, Scotland's Rural College). In addition, a bronchoalveolar lavage was performed for testing for bovine tuberculosis (*M. bovis*), although the disease has not been reported in beavers. Lavage fluid was submitted for standard mycobacterial culture and examined cytologically, including acid-fast staining for acid fast/mycobacterial organisms (Veterinary Pathology, RDSVS, University of Edinburgh).

An abdominal ultrasound examination was performed, with specific attention to the liver, to detect any abnormalities that could be indicators of *Em*. A 2-5MHz frequency convex abdominal ultrasound probe was used, and examinations recorded on a digital video recorder. Ultrasonography was performed by wetting the dense fur with 90% ethanol to allow adequate contact and good visualisation, in preference to clipping of fur, which it was considered may adversely affect the beavers waterproofing and thermal insulation when returned to the wild after testing.

A minimally invasive laparoscopic examination of the abdominal cavity was performed in the four adult animals to assess the liver and abdominal viscera for any signs of *Em* or other pathology not evident on ultrasonography, and physical examination. The fur and skin in the ventral midline region of the umbilicus was thoroughly cleaned and disinfected with a dilute chlorhexidine or dilute povidone/iodine based surgical scrub, followed by the application of surgical ethanol. A 6mm skin incision was made and the underlying ventral muscles blunt dissected to allow open access placement of a blunt trocar and 5mm cannula. The abdomen was insufflated with 8-10mmHg pressure using medical grade carbon dioxide. A five millimetre, 30 degree, 30cm paediatric laparoscope was inserted and the abdomen fully examined, with specific attention to the liver. The animal was repositioned in left and right lateral recumbency to allow movement of the viscera, and visualisation of all organ surfaces. At the end of the minimally invasive laparoscopic examination, the abdomen was deflated, the cannula removed and the muscle and skin were closed with absorbable monofilament Poliglecaprone suture material in two

layers. The skin closure was performed with a buried absorbable intradermal suture placement. Tissue adhesive was applied to the small skin incision wound to aid in immediate post-operative waterproofing. The resultant wound was approximately twice the size of a microchipping wound.

Genetics

DNA was extracted using a standard QIAGEN kit and normalised to 10ng/µl. Samples were run with a test developed at the WildGenes Laboratory of RZSS that consists of two mitochondrial Single Nucleotide Polymorphism markers (SNPs) that discriminate between *C. fiber* and *C. canadensis*. SNP analysis was conducted using an Applied Biosystems StepOne real-time thermal cycler and followed the standard amplification conditions for KASPar SNP probes as recommended by the manufacturer and as previously detailed[4]. The samples were run alongside two negative controls and positive controls for the two species (*C. fiber*, *C. canadensis*).

2.2.2 Ongoing screening and assessment of trial animals in the field

Individual passive identification tags were inserted subcutaneously in the dorsal neck region of each beaver to enable individual identification over the long-term.

2.3. Sourcing and screening for additional releases

2.3.1 Captive bred

Two captive bred individuals (1 female: 1 male) were sourced from two English collections. As these were proven captive bred, and not imported from outside the UK, they therefore had no opportunity to be exposed or acquire non-native pathogens, namely *Em* or tularaemia. Health screening did not involve a general anaesthetic and therefore various diagnostic screening including radiographs were not undertaken. This also meant no checks for malocclusion or signs of dental disease. However, as both individuals had been held in captivity with no reports of feeding issues, no concerns were presumed.

A physical examination was undertaken of both individuals. Weight was measured and body score assessed according to the standard rodent body scoring system. Each beaver was scanned for the presence of an identity microchip, and if not present beavers were microchipped in the inter-scapular region to allow future identification.

Blood and faecal samples were collected. More specific disease screening included full haematology and serum biochemistry as a general assessment of each beaver's general state of health (SAC Consulting Veterinary Services, Scotland's Rural College). Further specific serological testing was performed as follows: European *Leptospira* serovars 1-6 using the microscopic agglutination test (MAT) (APHA, Weybridge). Faecal samples underwent flotation with saturated salt solution for nematodes and sedimentation for trematodes, as well as microscopy for coccidia, *Cryptosporidium* spp., and *Giardia* spp. Standard microbiological culture for bacterial enteric pathogens, including enriched media for *Salmonella* and *Campylobacter* spp. was performed (SAC Consulting Veterinary Services, Scotland's Rural College).

2.3.2 Scottish wild caught

Two Scottish born animals from a conflict site identified through the Scottish Natural Heritage Beaver Mitigation Scheme. They were removed under trapping licence issued to RCP.

3. Findings

3.1. Pre-release health screening of original animals

Veterinary

All animals were physically healthy and presented no obvious deformities, discharge or obvious signs of disease. Evidence of previously healed wounds on all adult tails, and the hind foot of one adult female were observed, indicating historic injuries, most likely as a result previous territorial disputes. This is common and to be expected for this species. The missing hind toe of the adult female could also be indicative of historic trapping and/or transportation injury.

All beavers were deemed in good to very good body condition, with scores of 3 to 4, and defined as normal to good given time of year and age class. This was determined through examination of fat coverage of vertebrae and dorsal pelvis, and tail condition (thickness and lack of prominent tail arches). Sex confirmation, through examination of anal gland secretion and extended nipples, indicated that two adult females and two males were present, with the yearling sexed as female. Estimation of age class was made according to time of year, weight and body dimensions. The male 'adults' were considered to be smaller than the adult females, although their body dimensions could classify them as adults or mature sub-adults as a minimum. All beavers were tagged with passive transponder tags for individual identification.

General haematological parameters were judged against previously established normal published values for the Eurasian beaver (Girling *et al.*, 2015). All were largely unremarkable and no haemoparasites were recorded. The four adult individuals were screened for *Em* and all were found negative on serology, ultrasound and laparoscopic examination. All beavers were negative for tularaemia, as determined through PCR of serum samples. One beaver tested positive on serology for exposure to *Leptospira* spp. (Serovar Javanica, titre 1/800), the remaining four tested negative. On analysis of lung washes on the four adult beavers, all beavers were negative for acid fast/mycobacterial organisms and there was no evidence of any inflammatory lung response. Bacteriology screening for *Salmonella* and Johne's disease (*Mycobacterium avium* subsp. *paratuberculosis*) were negative. Parasitology was unremarkable with no evidence of *Cryptosporidium* spp., *Giardia* spp. or lungworm. Nematode and Coccidial oocyst counts were <50/gram faeces and therefore below the detectable threshold. Fluke eggs were only detected in D1 in which atypical eggs were seen, which are most likely to be *S. subtriquetrus* (beaver intestinal fluke), with no fluke eggs detected in any of the remaining individuals.

Genetics

Species Identification - The beavers all clustered genetically with *C. fiber*. One sample failed at one of the markers, however it clustered clearly with *C. fiber* at the second marker. Low DNA concentration is this sample is the likely cause of failure for this first SNP.

Population Origin and Genetic Diversity – Samples were run with a test developed at the WildGenes Laboratory of the Royal Zoological Society of Scotland that consists of 29 nuclear Single Nucleotide Polymorphism markers (SNPs) that discriminate between different populations of beaver. SNP analysis was conducted using an Applied Biosystems StepOne real-time thermal cycler and followed the standard amplification conditions for TaqMan SNP probes as recommended by the manufacturer.

Samples were run with 2 negative and 7 positive controls, and compared to a reference dataset of 307 beavers of known population origin using the population assignment program GenClass2. All animals assigned with high probability to either Bavarian or Baden-Württemberg populations. These are German populations of mixed reintroduced origin. This strongly suggest that the beavers came from German mixed reintroduced stock. The animals assign with very low probability to France, Norway and a number of Eastern European/ Eurasian populations. The animals had a lower level of heterozygosity (He) than the reference source populations that they matched to. The value of He for Devon was 0.339 (with a standard error of 0.03) compared to values of 0.453 (s.e. 0.016) and 0.478 (s.e. 0.005) for Baden-Württemberg and Bavaria respectively (values calculated using comparative loci).

Family Relationships- Using the same 29 nuclear Single Nucleotide Polymorphism markers (SNPs) as used for population assignment, potential familial relationships were examined by calculating estimates of pairwise molecular relatedness (in the software Genalex). Likely family combination were also examined by eye to exclude possible combinations and their statistical likelihood examined in the software Colony. These markers have been shown to have sufficient power to examine issues of relatedness in the target populations. Pair-wise relatedness between all individuals was high and was approximately equivalent to being between the first order (e.g. parent-offspring/full sib) and the second order (e.g half-sib) relationship level. There is a lot of statistical noise around these estimates, so it is not possible to use these estimates to be sure of the exact degree of relatedness (other than being very close). Examination of the genotypes involved in the different possible parent-offspring relationships (that were likely between the beavers based on their age and sex) was able to rule out or suggest a number of potential parent-offspring combinations. None of the potential relationships could be confirmed with a high degree of statistical certainty due to close degree of relatedness of the individuals (i.e. another form of close relationship is also theoretically possible).

3.2. General ongoing disease screening

Number of trapped individuals (note some individuals were trapped multiple times).

- 2015 five individuals (original trapped animals)
- 2016 three individuals
- 2017 six individuals
- 2018 seventeen individuals
- 2019 twelve individuals

Figure 3. Adult beaver feeding from non-set trap to encourage use and monitor which individuals coming to trap.

3.2.1 2016/2017

Blood samples from two beavers showed seroconversion to *Leptospira* spp. Adult male (microchip ending 5847) produced a positive titre for serovars *Australis* (1/800) and *Bratislava* (1/400) on MAT testing/ Adult female (microchip ending 5874) produced a positive titre for serovar *Hardjo-Prajitno* (1/100) on MAT testing.

3.2.2 2017/2018

Beaver	Sex	Haematology	Faecal parasites	Faecal bacteria
6734	M	No abnormalities, evidence of anaemia or inflammatory disease).	No evidence of parasites: no oocysts of *Cryptosporidium* spp.; <50opg for coccidia; negative for *Giardia* spp. and fluke	Negative for *Yersinia* spp. and *Salmonella* spp.
6183	F	No abnormalities, evidence of anaemia or inflammatory disease).	No evidence of parasites: no oocysts of *Cryptosporidium* spp.; <50opg for coccidia; negative for *Giardia* spp. Positive for liver fluke (*Fasciola hepatica*)	Negative for *Yersinia* spp. and *Salmonella* spp.
0519	F	No abnormalities, evidence of anaemia or inflammatory disease). Mildly elevated creatinine kinase (possibly associated with capture) and potassium levels (associated with haemolysis/age of blood sample)	No evidence of parasites: no oocysts of *Cryptosporidium* spp.; <50opg for coccidia; negative for *Giardia* spp. and fluke, <50epg for *Moniezia*, *Strongyles*, *Strongyloides*, *Nematodirus* and *Trichuris* spp., *Giardia* spp., fluke or lungworm	Negative for *Yersinia* spp., Campylobacter spp. and *Salmonella* spp. Negative for Johne's disease (*Mycobacterium avium* subsp. *paratuberculosis*) disease on Ziehl Nielson smear
9854		*Insufficient samples*	No evidence of parasites: no oocysts of *Cryptosporidium* spp.; <50opg for coccidia; negative for *Giardia* spp., fluke and nematodes including lungworm	Negative for *Clostridium perfringens*, *Yersinia* spp., Campylobacter spp. and *Salmonella* spp. Negative for Johne's disease (*Mycobacterium avium* subsp. *paratuberculosis*) disease on Ziehl Nielson smear
0829		*Insufficient samples*	No evidence of parasites: no oocysts of *Cryptosporidium* spp.; <50opg for coccidia; negative for *Giardia* spp., fluke and nematodes including lungworm	Negative for *Clostridium perfringens*, *Yersinia* spp., Campylobacter spp. and *Salmonella* spp. Negative for Johne's disease (*Mycobacterium avium* subsp. *paratuberculosis*) disease on Ziehl Nielson smear

Summary of veterinary results 2017/2018

The only abnormal result identified was the one positive *Fasciola hepatica* (liver fluke) sample from 6183 female beaver in January 2017. *F. hepatica* infection has been reported in two Eurasian beavers out of 20 in a short communication (Shimalov & Shimalov, 2000). In this communication, the condition was diagnosed at post-mortem on liver examination and eggs were present in the faeces although no

attempt to differentiate the eggs from the intestinal trematode *S. subtrequetrus* was made by PCR, bringing into doubt whether the infections were truly patent. Infection in coypu (*Myocaster coypus*) though did suggest that this semi-aquatic rodent was capable of developing a patent infection and potentially spreading it to other susceptible species via faeces (Dracz *et al*., 2016). It is possible that the reported result of *F. hepatica* in this case was the result of a true infection of that individual, but the possibility of misidentification with *S. subtrequetrus* is a real possibility as PCR methods were not applied by the laboratory.

Otherwise, the results of blood sampling and faecal analysis did not demonstrate any evidence of infectious or degenerative disease, although it should be noted insufficient blood samples prohibited further testing.

3.2.3 2018/2019

Over the winter period (2018/2019) as many of the beavers residing within the River Otter were trapped as possible to enable tagging and sample collection. Health screening followed reduced screening requirements for British born beavers, focusing on native parasite and diseases of concern. General body condition assessments were undertaken to ensure beavers were capable of coping with the restoration process (Animal Welfare Act, 2006).

Blood and faecal samples were taken from Eurasian beavers around the River Otter, Devon, under license by the Devon Wildlife Trust (DWT) over the period December 2018 to March 2019. Samples were analysed for health and a range of pathogens by staff at the RZSS. A total of 15 animals had faecal samples analysed and 9 had blood samples analysed by RZSS.

Faecal analysis - No evidence of *Giardia* spp., *coccidia* (including *Cryptosporidium* spp.), nematodes, cestodes or trematodes were identified in samples tested from 15 beavers. No evidence of *Salmonella*, Shigella, or *Campylobacter* spp. was found in samples tested from 15 beavers. One beaver out of 15 was positive for *Yersinia* spp. which was speciated to *Yersinia frederiksenii*. This individual was in extremely good body condition, and is a known older breeding female, in the early stages of pregnancy at time of examination. This bacterium is not considered a serious pathogen of wildlife or humans.

Blood analysis - Full haematological analysis was only carried out in one animal sampled at this time due to poor preservation of cells, but smear analysis on a further three beavers sampled did not demonstrate any obvious abnormal white cell differential suggesting there was no evidence of inflammatory disease in those sampled. Those samples suitable for biochemical analysis (n=8) were within previously published values for Eurasian beavers suggesting overall good-health (Girling *et al*., 2015).

Six animals were tested for evidence of *Leptospira* spp. antibodies using a previously utilised micro-agglutination test (MAT) to pools 1-6 and all were negative.

3.3. General body condition

There was no cause for concern in relation to body condition throughout the trial, including released animals and any ongoing trapped/re-trapped trial animals. The only exception was one kit trapped in the December of that year it was born that had less fat coverage along the spine and pelvis but still had a good body weight. This individual was still fit with no other obvious health concerns so was released back into its family group. In fact, all animals were in good to very good body condition, with appropriate and acceptable weight ranges for age class trapped. Adults all had body weights of 19- 25+kg. Tail and

pelvic region fat coverage was good and presumed to only increase into the summer period. Indeed, the fact that one breeding female had documented litters of 4 and 5 kits is highly indicative that excess resources exist in this landscape to support good body conditions over the winter period and high litter sizes.

3.4. Screening of additional animals

3.4.1 Captive bred

For both individuals, no enteric parasites or significant bacterial pathogens were isolated. Eggs of *S. subtrequetris*, the beaver intestinal fluke were identified in one beaver. Blood results were unremarkable for both except for mild elevation of liver parameters in one individual, though these were not considered significant. Insufficient serum was available for *Leptospira* spp. serology or serum protein electrophoresis in one beaver whilst the other was positive on serology for *Leptospira* pool 6 (positive for *Hardjo-Prajitno* at 1/100) suggesting exposure to this organism. No evidence of an inflammatory white cell response or electrophoretic response was observed and renal parameters were within normal bounds suggesting no evidence of an active inflammatory response.

3.4.2 Scottish wild caught

One male sub-adult (~1.5years) live trapped from a conflict site in Scotland underwent basic physical examination and sample collection at Battle Flatts Veterinary Clinic. This individual weighed 12kg with body condition score of 3/5. There were no obvious issues on clinical examination. Blood and faecal samples were sent to CTDS Laboratory, no significant abnormalities were noted, this individual was negative for *Leptospirosis*, *Giardia*, *Salmonella*, *Cryptosporidium* spp. and no indication of any faecal helminth parasites.

One female subadult (~2years) live trapped from a conflict site in Scotland underwent basic physical examination and sample collection at RSPCA West Hatch. This individual weighed 15kg with body condition score of 3.5/5. There were no obvious issues on clinical examination. Blood and faecal samples were sent to Idexx Laboratory and no significant abnormalities were noted. This individual was negative for *Leptospirosis*, *Giardia*, *Salmonella*, *Cryptosporidium* spp. and there was no indication of any faecal helminth parasites.

Figure 4a and b. Minor tail scarring from beavers trapped 2019, most likely indicating some level of family / territorial disputes.

4. Discussion

4.1. Pre-release screening – original animals and additional releases

4.1.1 Health and body condition

The initial founder population was in good health and body condition at the time of health screening in 2015. No evidence of serious zoonotic disease (*Em*, *F. tularensis*, *Giardia* spp. etc.) was evident in any animal despite extensive testing procedures.

Throughout the course of the trial all trapping occurred from December to early April, the time when beavers are experiencing or coming out of a winter period and typically at their lowest body condition after low vegetation growth. Regardless of this weights and body conditions were all good-to very good, clearly indicating that beavers are surviving in good health, easily obtaining food resources and well adapted to this landscape. High kit numbers and survival of individuals year to year also suggests beavers are not highly challenged in this area.

Figure 5. Pre-release blood sampling with local veterinary team.

4.1.2 Genetics

All beavers trapped were presumed to be Eurasian, established through genetic screening and physical sampling (AGS) of trapped animals. Over the trial period there has been no cause for suspicion of North American beavers being present. The five original beavers genetically sampled were most likely to be of German reintroduced population origin and were highly genetically similar to reference samples held for Bavaria and Baden-Württemberg populations. Values of genetic diversity in the River Otter population were lower than these possible source populations. Examination of genetic relatedness revealed that the original beavers were closely related, consistent to belonging to a single-family group. Reason to release additional animals throughout the trial with the aim to increase the genetic diversity of this population was therefore established.

4.2. Ongoing monitoring throughout the trial

All beavers tested throughout the five years of the trial continued to demonstrate good clinical health. No signs of serious zoonotic disease (e.g. *Em*, tuberculosis, *Salmonella* or *Giardia* spp.) were noted in any animal. Some individuals showed signs of exposure and serological response to potential pathogens such as *Leptospira* spp., but interestingly in the latter parts of the trial, all animals tested had become serologically negative, suggesting waning of the immunological response. No beaver showed signs of kidney or liver disease which has been associated with infections with *Leptospira* spp. disease in other mammals. Laboratory results for one beaver suggest evidence of persistent liver fluke (*F. hepatica*) infection. However, there is some questioning of this result as PCR for species identification was not undertaken. This individual did not show any clinical signs of disease and other beavers in the trial remain unaffected. All veterinary testing so far suggests that the beavers in the River Otter Trial remain clinically healthy and appear to not be a source of significant infectious disease.

4.3. Screening limitations

Some limitations with veterinary screening have to be acknowledged as not all animals were able to be screened each year. Technical difficulties in collecting and preserving samples in the field meant that some animals were not able to be screened for all diseases. However, the majority of animals were sampled throughout the trial period, and particularly where significant, zoonotic conditions were concerned (e.g. *Giardia* spp., *Salmonella* spp.), they were tested and found to be free of disease.

4.4. Potential health and welfare impacts on restricted genetic diversity

The possible impacts of a restricted founder base on the health of the five-year trial are hard to assess. Given that the population is currently determined to be in a healthy state it may be unlikely that any measurable fitness effects of inbreeding would appear over the five-year lifespan of the trial, and even if they did, it would be difficult to attribute them to inbreeding versus other factors.

Evidence of longer-term impacts of inbreeding within less genetically diverse Eurasian beaver populations is mixed. Higher instances of inbreeding depression and phenotypic abnormalities have been reported in less genetically diverse populations (Halley, 2011). However, it is not entirely clear that these have not been caused by other confounding factors (see Rosell *et al*., 2012 for discussion). Differences in fecundity have been reported between single refugia and 'mixed' populations, with mixed populations displaying greater genetic diversity along with higher fecundity rates (Halley, 2011). Again, fecundity can be affected by numerous factors including food availability and population density (Payne, 1984). On the other hand, beaver populations have successfully recovered and been restored from very small numbers of founder animals. For example, Swedish populations have recovered from ~11 breeding Norwegian females (with Norwegian populations themselves identified as having restricted genetic diversity recovering from ~120 individuals). Both Swedish and Norwegian populations have recovered without a common display of the more typical abnormalities associated with inbreeding (e.g. dental abnormalities, cleft palates, polydactyla etc) (Parker *et al*., 2012; Rosell *et al*., 2012). Nevertheless, such evidence is anecdotal and does not mean that more diverse population would not have a better chance of success over longer time scales. Within animal species there is generally compelling evidence that inbreeding leads to inbreeding depression in the long run and perhaps more importantly a lack of adaptive potential (i.e a loss of ability of the population to adapt to future challenges, be these due to disease or environmental obstacles). The arguments laid out in Senn *et al*. (2014) discuss the benefits of genetically diverse founding stock for beaver reintroductions in more detail.

Finally, should a future reintroduction be approved, attempting to construct a more robust and diverse founder base would be strongly recommended. Ensuring the reduction of inbreeding (particularly between parent-offspring and sibling-sibling matings) is considered best practice in species restoration projects. The IUCN Reintroduction Guidelines require the selection of an adequately diverse founder base (IUCN/SSC, 2013). Although this is a trial project, perhaps it is best if managed with this in mind. Should the trial be eventually allowed to progress to a full-scale reintroduction, these original individuals and their descendants would almost certainly be part of the founding stock. Care should also be taken in using this population as founders for other beaver populations in the near future, as individuals are still exhibiting low genetic diversity and additional animals have not bred over multiple generations at this stage.

4.5. Recommendations for future monitoring

The level of post-release health monitoring should reflect the assessed level of risk. As recommended by the IUCN Reintroduction Guidelines, there should be a level of assessment to determine to what extent an establishing population is experiencing disease, adverse welfare conditions or mortality (IUCN/SSC, 2013). Post-mortem examination of any recovered cadavers should provide an opportunity to determine body condition, adaptation to release and screen for diseases and parasites according to previous recommendations (Goodman *et al.*, 2012; Campbell-Palmer & Rosell, 2013). Any evidence of wildlife or domestic animal disease in release areas related to those pathogens previously associated with beavers should be fully investigated. Future genetic sampling to measure the success of the addition of unrelated individuals and the genetic diversity of the population could provide a useful measure of this proactive approach and inform future beaver population management recommendations.

Figure 6. Infield ongoing screening of trial animals, adult male re-released after trapping.

5. Conclusions

Overall, the health of the beavers on the River Otter appears to have been consistently good throughout the five years of the study. No evidence of significant zoonotic disease has been apparent. Seroconversion to *Leptospira* spp. has been seen but without clinical signs of disease and subsequent serology has shown a waning antibody titre suggesting the individuals are not persistently infected. These findings contrast with some case reports in the literature that suggest beavers are highly susceptible to disease with *Leptospira* spp. infections, with mortalities likely, suggesting other factors may have been at work in those published cases (Nolet *et al*., 1997; Marreros *et al*., 2018).

Ongoing health assessments at a reduced intensity are advised to monitor for any signs of emerging diseases, particularly as the population grows. Post mortems should be carried out where possible on any animal recovered as these allow a more thorough and full assessment of the presence or absence of significant pathogens.

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Appendix 1. – Disease Risk Assessment

Table 1. Summarised disease risk assessment for pathogens mentioned in report text.

Agent	Reported in *Castor fiber*?	Reported in other rodents?	Zoonosis?	Risk band pre-management action	Present in the UK?	Risk management actions	Risk band post-management action
Cryptosporidium spp.	Infection reported in Poland (Bajer *et al*. 1997) and UK (Goodman *et al*. 2012)	Common in rodent species, such as *C. muris* in mice and *C. wrairii* in guinea pigs (*Cavia porcellus*), neither of which are thought to be zoonotic (Bajer *et al*. 1997)	Yes *C. parvum*	High	Yes	Faecal parasitology (+/- acid fast staining) or RT-PCR	Low
Echinococcus multilocularis	Found in a captive animal in the UK imported from the wild in Bavaria, Germany (Barlow *et al*. 2011)	Reported in field and water voles; particularly common in endemic areas (Miller *et al*. 2016)	Yes	High	No (except captive beaver reported by Barlow *et al*. 2011)	Use UK captive-bred or British born animals as source population. Alternatively carry out testing protocol as described by Campbell-Palmer *et al*. (2015b) and Gottstein *et al*. (2014)	Low
Fasciola hepatica	Found in 2 of 20 beavers examined (Shimalov & Shimalov 2000)	Recorded in coypu (*Myocastor coypus*) (Dracz *et al*. 2016)	No	High	Yes	Faecal parasitology	N/A
Giardia spp.	Yes – incidence 0-8% in the wild in one study in Poland (Bajer *et al*. 2008) Not detected in post reintroduction site monitoring at SBT (Mackie 2014)	Common in rodent species (Olsen & Buret, 2001)	Yes	High	Yes	Faecal parasitology	Low

Stichorchis subtriquetrus (Beaver fluke)	Yes – Goodman *et al*. (2012), Campbell-Palmer *et al*. (2013), Máca *et al*. (2015)	No	No	Low	Yes	N/A	Low
Travassosius rufus (Beaver stomach nematode)	Prevalence rates of 82% and 93%, have been recorded in Czech (Máca *et al.* 2015) and Polish beavers respectively (Drózdz *et al*. 2004)	No	No	Med	No	Faecal parasitology	Low
Platypsyllus castoris	Common on Eurasian beavers throughout its range. Found on Scottish born beavers (Duff *et al*. 2013)	Yes – Peck (2006)	No	Low	No	NA	Low
Campylobacter spp.	No	Reported in guinea pigs bred for food in South America (Graham *et al*. 2016). 4% incidence of *C. jejuni* by culture in wild rodents (Backhans *et al*. 2013)	Yes	Low	Yes	Na	Low
Leptospira spp.	Present in Scottish beavers and cause of translocation mortalities (Nolet *et al*. 1997, Goodman *et al*. 2012, Goodman *et al*. 2017, Marreros *et al.* 2018)	Widely reported in captive and wild rodents (Yarto-Jaramillo 2015, Gelling *et al*. 2015)	Yes	High	Yes	Serological testing	Low
Mycobacterium avium subspp paratuberculosis (Johne's disease)	No	No	Yes	Low	Yes	NA	Low

Salmonella spp.	Salmonella spp. have been identified in wild Eurasian beavers in Norway (Rosell et al. 2001), Germany and Russia (Romasov 1992) but not speciated	Common in Muridae and Cricetidae. Reports of *S. enterica* serotype typhimurium, *S. enteritidis* and *S. typhimurium* are common in these species (Yarto-Jaramillo 2015)	Yes	High	Yes	Faecal bacterial culture and/ or use British born animals as source population (Campbell-Palmer *et al*. 2015a)	Low
Hantavirus	No (no evidence in survey by Girling *et al*. 2019)	Reported in the UK in Muridae – principally the brown rat and the bank vole (McElhinney *et al*. 2016, Bennett *et al*. 2010)	Yes	Low	Yes	N/A	Low
Rabies	No	Yes – but not present in the UK	Yes	High	No	Use UK captive-bred or British born animals as source population. Or Rabies quarantine imports	Low