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# ESTABLISHMENT OF A HEALTH SURVEILLANCE PROGRAM FOR REINTRODUCTION OF THE EURASIAN BEAVER (*CASTOR FIBER*) INTO SCOTLAND

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ABSTRACT: In 2009 and 2010 16 Norwegian Eurasian beavers (*Castor fiber*) were reintroduced to Knapdale, Scotland as part of a 5-yr reintroduction trial (Scottish Beaver Trial). Despite numerous reintroduction programs throughout Europe there is no published information concerning recommended health surveillance during beaver reintroduction and only one publication describing causes of mortality. We describe the establishment of a health surveillance program based on International Union of Conservation of Nature (IUCN) and governmental guidelines, and report preliminary results based on the fecal and blood samples following the completion of the first stage of reintroduction. Animals underwent at least one general anesthetic to allow collection of fecal and blood samples and a thorough clinical examination. No bacterial enteric pathogens such as *Salmonella* spp., *Campylobacter* spp., or *Yersinia pseudotuberculosis* were isolated, nor were *Giardia* spp. or *Cryptosporidium* spp. However, numerous helminths including *Travassosius rufus* and *Stichorchis subtriquetrus* were detected. Five animals were positive for *Leptospira* antibodies. This included *Leptospira saxkoebing*, *Leptospira canicola*, *Leptospira copenhageni*, *Leptospira icterohaemorrhagiae*, *Leptospira autumnalis*, and *Leptospira javanica*. The highest loss of animals (20%) was during the statutory 6-mo rabies quarantine period. No common cause of death was determined. The rabies quarantine conditions were waived for four remaining animals, three of which were introduced to the wild successfully. The authors recommend the shortest possible quarantine period when introducing beavers, but allowing for the minimum recommended IUCN 35 days to allow for implementation of the initial stage of the health surveillance program, examination of animals, sample collection, and processing.

*Key words:* Anesthesia, *Castor fiber*, disease surveillance, Eurasian beaver, giardia, health, reintroduction, *Stichorchis subtriquetrus*.

## INTRODUCTION

A reintroduction program should include a feasibility study that looks at ecologic, environmental, and socioeconomic impact on the likelihood of success. As part of this, a health risk assessment with recommended health screens should be carried out, as the translocated animal is a biological package and therefore can potentially introduce new pathogens into the release area (Jessup, 1993; Cunningham, 1996; Kock et al., 2010). Health risk assessment is a rigorous application of common sense to evaluate whether important health-related risks are associated with a proposed activity, such as translocation of wild animals (Leighton, 2002).

There have been a few detailed publications on health protocols for translocating wildlife, such as the article on health protocols for translocation of free-ranging elk (Corn and Nettles, 2001). Mathews et al. (2006) argue that baseline data on the health status of reintroduced animals and extant populations are rarely published.

Translocation and reintroduction of geographically extinct populations is increasingly used as a conservation tool. The Eurasian beaver (*Castor fiber*) probably became extinct in Scotland sometime between the 15th and 16th centuries because of hunting for pelt, meat, and castoreum (Kitchener and Conroy, 1997). It is believed that the original Eurasian

beaver found in Scotland more closely resembles the Scandinavian Eurasian wild beaver (Kitchener and Conroy, 1997). The first known beaver reintroduction program, in 1922, was in Sweden, with animals from Telemark, Norway. Since then, at least 203 translocations to distinct sites have been recorded outside the former Soviet Union (Halley, 2011).

During 2009 and 2010, three Eurasian beaver families (three animals in one and two with four members), two pairs and a single replacement animal for one of these pairs were reintroduced to Knapdale (latitude 56°2′10.7478″, longitude −5°35′11.6376″) in the west of Scotland, as part of a 5-yr scientific reintroduction trial under a Scottish government license (Scottish National Heritage, 2009).

Despite numerous Eurasian beaver reintroduction programs little has been published on the health surveillance, disease, and mortality associated with these reintroductions. We describe the establishment of a beaver health surveillance program based on International Union of Conservation of Nature (IUCN) guidelines and report preliminary results.

## MATERIALS AND METHODS

Beavers were trapped in freshwater river systems in the Skien and Nome municipalities of Telemark, Norway. Potential sites and families were identified before trapping. In the field beavers were trapped with the use of a landing net, which allows selective trapping and enables caught animals to be transferred easily from the trapping net into a hessian sack for handling (Rosell and Hovde, 2001). Twenty-nine wild-caught beavers were initially quarantined for 1 mo in holding facilities at Lunde, Telemark, Norway. No beavers were injured during trapping. Each family was held in a separate room with walls lined with sheer sheet metal (1 m high) to prevent gnawing and reduce injuries to their paws. Concrete flooring was covered with a deep layer of sawdust and straw. Each holding pen contained a water pool that was changed every other day, spoiled substrate was removed daily. Beavers were fed on local browse, apples, and carrots. One kit died in quarantine; the cause of death was not established. These facilities and animal welfare procedures followed Norwegian Food and Safety Authority regulations. On arrival to the United Kingdom they were placed in quarantine facilities for 6 mo to comply with UK rabies legislation. They were housed in purpose-built facilities in Broadwoodwidger in Devon between February 2008 (first families) and May 2009 (second families). Each beaver family was placed within a metal-lined holding pen with a heat lamp and access to fresh bedding, water, and food. Water was changed every other day, bedding was added as appropriate, and the beavers were fed on a diet of carrots, apples, fodder beet, and willow.

Later, the rabies quarantine requirement was reduced to under a month in the United Kingdom for four animals, following Norwegian state veterinarian certification that the beavers came from rabies-free areas. Of the 29 animals, 6 died during the UK quarantine period, 16 animals were released to Knapdale, and the rest remained at Royal Zoological Society of Scotland (RZSS) animal collections. These animals were intended by RZSS as holding stock for their collections at Edinburgh Zoo and Highland Wildlife Park, as it may have been necessary to draw upon these animals if replacement animals were required.

The prerelease health screens and postrelease monitoring protocols in the Scottish reintroduction program were based on the IUCN guidelines (Woodford, 2000) and incorporated governmental (Department of Environment, Food and Rural Affairs) and public health concerns such as rabies and giardia. A literature search on reported diseases in captive and wild beavers was also used to formulate these protocols with the use of CAB ABSTRACTS, BIOSIS, and MEDLINE databases.

All the animals underwent screening at least once prior to release. Animals that were in a holding facility of the RZSS, following discharge from rabies quarantine and prior to release, underwent a second screening. The screening program, performed by a veterinarian, included a comprehensive clinical examination of each individual under general anesthesia. Anesthesia protocols varied between the veterinarians and included inhalation anesthetics or injectable agents. Beavers in captivity were either netted or walked into a travel crate, from which they were transferred into a hessian sack for handling or hand injected with sedative for full clinical health screen and sample collection. Anesthesia was achieved with the use of isoflurane alone via a face mask or by the administration of injectable anesthetic agents followed by administration of isoflurane via a face mask. Isoflurane gaseous anesthetic was administered at 3–4%

for induction and 1–2% for maintenance of anesthesia with an oxygen flow rate of 2 L. Injectable agents were a combination of 0.2 mg/kg diazepam and 10–12.5 mg/kg ketamine or 0.06–0.08 mg/kg medetomidine and 6.6–9.5 mg/kg ketamine administered by intramuscular injection after manual restraint.

The clinical examination followed normal veterinary practices and included examination of the head (eyes, ears, and teeth), skin, fur, and tail (for ectoparasites and injuries), abdominal palpation, auscultation, and cardiac evaluation. All animals were weighed and a subcutaneous microchip was inserted, or previous placement confirmed, under the skin at the back of the neck. The sex of each animal was also confirmed by one or more methods, such as palpation of the baculum (os penis; Osborn, 1955) or the color and viscosity of the anal gland secretions (Rosell and Sun, 1999).

Blood samples (2 ml in $K_2$ EDTA and 10 ml whole blood) were obtained from conscious animals trapped and restrained in a hessian sack (Rosell and Hovde, 2001) or anesthetized animals, via the ventral tail vein with a 21G/3.8-cm needle attached to a 5-ml syringe. Serum samples were submitted for tularemia (*Francisella tularensis*) enzyme-linked immunosorbent assay (ELISA: Greendale Veterinary Diagnostics limited, Woking, UK) or polymerase chain reaction (PCR; National Veterinary Institute, Oslo, Norway) and European *Leptospira* serovars using the microscopic agglutination test (MAT) at the Veterinary Laboratory Agency in Weybridge, United Kingdom. Sixteen serum samples were tested for antibodies via agglutination tests for antibodies to *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* (Pinmoore Animal Laboratory Services Ltd., Tarporley, UK). These two pathogens were included in a bacterial fecal culture screen for 13 animals. A hematology and biochemistry panel was run for each animal. When possible, additional serum samples were stored at −40 C for future or retrospective screening if required. Rectal fecal samples were collected under general anesthesia. Fecal samples were submitted for bacterial enteric pathogens. *Salmonella* spp., *Campylobacter* spp., *Clostridium* spp., and *Yersinia* spp. were specifically requested.

Fecal samples also underwent floatation with saturated salt solution for nematodes and sedimentation for trematodes as described in the RVC/FAO Guide to Veterinary Diagnostic Parasitology (Royal Veterinary College [RVC], 2010). Fecal samples were screened for *Giardia* spp. with the use of a SNAP® Giardia immunoassay kit (IDDEX Laboratories, Whetherby, UK). A selective *Cryptosporidium* spp. staining kit and direct microscopy was used for detection of cysts.

Once the beavers were released, health surveillance of beavers by field workers consists of annual trapping of individual animals and regular observations of body condition and behavior. Depending on the habitat animals were trapped on land by Bavarian traps or by boat with the use of landing nets. For nonanesthetized individuals, the beaver's head was kept pressed into the corner of the hessian sack; restraint was possible through either carefully straddling the beaver and restricting movement through gentle pressure from legs or lying alongside the animal with one arm across the body. In both methods the handler faced the rear end, holding on to the base of the tail or behind the back legs to discourage the beaver reversing out of the sack. The head remained in the corner of the sack at all times, and gentle pressure was applied from above to prevent the beaver from rearing upwards. Fecal samples were collected easily by feeling just behind the anus for internal lumps which indicated the presence of feces in the colon. The sampler then gently applied pressure on both sides at the same time pulling toward the sampler until feces exited the anus. Blood samples can be collected from a correctly restrained, nonanesthetized beaver from the underside of the mid vein of the tail.

Animals were trapped at least once per year and weighed, body measurements taken, and biological samples collected, including fecal samples, which were screened again for parasite and enteric pathogens. If a veterinarian was present, a blood sample was obtained for *Leptospira* spp. screening, hematology, and biochemistry. Animals that died during the trial reintroduction period were subjected to full postmortem examination.

## RESULTS

All animals were negative for zoonotic enteric bacterial pathogens, including *Campylobacter* spp., *Salmonella*, and *Yersinia* spp. Neither *Giardia* spp. nor *Cryptosporidium* spp. were detected. None of the 29 animals were antibody positive for tularemia. Five animals were positive for *Leptospira* antibodies, included *Leptospira saxkoebing*, *Leptospira canicola*, *Leptospira copenhageni*, *Leptospira icterohaemorrhagiae*, *Leptospira autumnalis*, and *Leptospira*

*javanica.* Numerous helminths were identified in the fecal samples. *Travassosius rufus* and the fluke (*Stichorchis subtriquetrus*) were the most commonly identified parasites (eight animals). Hematology and biochemistry results were within normal reference ranges for European beavers (Girling et al., unpubl.).

Poor body condition and dental abnormalities may lead to postponement of release or exclusion from the trial. The release of one pair was postponed because the male had pulp exposure of the upper right incisor and inflammation of the upper lip. On a follow-up clinical examination 1 mo later the incisors were deemed to have grown back to normal and the animals were released.

Six animals died during the 6-mo quarantine period. No common cause of death was identified. Four animals had signs of infection or inflammation. An adult female died with an infected anal gland and colitis, but no pathogens were isolated from either organ. A male kit died with inflammatory reaction in the small intestines and *Escherichia coli* was isolated from the intestine and liver. An adult female died with meningitis/encephalitis with no significant bacterial growth. An adult male died with osteomyelitis of the coccygeal vertebra (*Arcanobacterium pyogenes*) and liver necrosis. In two animals no primary cause could be identified. These were an autolysed female kit from which no pathogens were isolated and a juvenile female in poor body condition with severe intestinal parasitism. Two animals died following release to Knapdale. The first was a juvenile male with lung, liver, and kidney congestion suggestive of subacute circulatory failure, but with no evidence of infection or degenerative disease. The second was an adult male in poor body condition and autolysis of the carcass, which prohibited histologic and microbiologic examination.

## DISCUSSION

Corn and Nettles (2001) developed a five-component protocol to reduce the introduction of infectious agents in translocated animals. The first step is to evaluate the health status of the source population. Norwegian beavers are of lower risk for introduction of rabies (MacDonald et al., 2011) and *Echinococcus multilocularis* (Vitenskapskomiteen for Mattrygghet [VKM], 2012). No commercial antemortem tests exist for either. All animals were negative for zoonotic enteric pathogens, which seems to be in line with results from an investigation of waterborne pathogens from fecal samples in beavers in southeast Norway (Rosell et al., 2001).

The second step is to quarantine the animals. In our program the animals were placed in quarantine on arrival. The length of quarantine was dictated by governmental animal health departments. In the United Kingdom this is 6 mo for rabies. If this is waived a quarantine period of 35 days is recommended by the IUCN to allow processing of diagnostic samples as described in Table 1.

The third step is physical examination of the animals and diagnostic testing. We recommend this be done under a general anesthetic in beavers to allow for thorough physical examination, including dentition. The list of diagnostic tests is based on IUCN guidelines and includes diseases of zoonotic potential and diseases that currently do not occur in the United Kingdom.

None of the 29 animals were antibody positive for *F. tularensis*, nor were any signs of rabies observed. Tularemia does not occur naturally in the United Kingdom, and the United Kingdom has been rabies-free since 1970 (Department for Environment Food and Rural Affairs [DEFRA], 2011). Numerous pathogens associated with rodents are not included in the IUCN guidelines; for example, *E. multilocularis* has been reported in Eurasian beavers in Switzerland (Janovski et al., 2002), but not on mainland Norway, and in one captive beaver in the United Kingdom (Barlow et al., 2011). *Mycobacterium microti* (Cavanagh et al., 2002),

TABLE 1. Summary of infectious agents to be considered for screening and their impact on potential beaver reintroduction of Eurasian beavers (*Castor fiber*) into the United Kingdom.

| Agent | Test | | Risk/status in the United Kingdom (UK) | Reference | Action if positive |
|---|---|---|---|---|---|
| | Sample[a] | Laboratory test[b] | | | |
| Bacterial | | | | | |
| *Campylobacter* spp. | Fecal | Bacterial culture | Endemic in the UK. | Brown et al. (2004) | Treat and retest |
| *Clostridium* spp. | Fecal | Bacterial culture | Endemic in the UK. | | Treat and retest |
| *Francisella tularensis* | Serology | PCR or ELISA | Risk dependent on source of animals. Does not occur in the UK. | | Exclude animal from reintroduction |
| *Leptospira* spp. | Serology | MAT | Endemic in the UK. | Salt and Little (1977) | Permit release of animal |
| *Salmonella* spp. | Fecal | Bacterial culture | Endemic in the UK. | Williams (1975) | Treat and retest |
| *Yersinia enterocolitica* *Yersinia pseudotuberculosis* | Fecal serum | Bacterial culture or AGT | Endemic in the UK. | | Treat and retest |
| Parasitic | | | | | |
| *Cryptosporidium parvum* | Fecal | Microscopy with selective staining | Endemic in the UK. | Quy et al. (1999) | Exclude animal from reintroduction |
| *Echinococcus multilocularis* | No commercial antemortem test available | | Risk dependent on source of animals. Low in animals from Norway but higher in animals of German origin. Does not occur in the UK. | Barlow et al. (2011) | Exclude animal from reintroduction |
| *Giardia* spp. | Fecal | Giardia immuno-assay kit | Endemic in the UK. | Batchelor et al. (2008), Hutchison et al. (2004), Smith et al. (2006) | Treat and retest |
| *Stichorchis subtriquestrus* | Fecal | Sedimentation technique | High prevalence reported but low morbidity/mortality. Beaver-specific parasite. No known effective treatment. | Ahlen (2001) | Permit release of animal |
| Viral | | | | | |
| *Rabies virus* | Necropsy of suspect cases | | Risk dependent on source of animals. Low in animals from Norway but higher in animals of German origin. Does not occur in the UK. | | Exclude animal from reintroduction |

[a] The serological techniques are not beaver specific.

[b] PCR = polymerase chain reaction, ELISA = enzyme-linked immunosorbent assay, MAT = microscopic agglutination test, AGT = antiglobulin test.

cowpox virus (Carslake et al., 2005), *Cryptosporidium parvum* (Quy et al., 1999), *Eimeria* spp. (Lewis and Ball, 1983), *Bartonella* (Birtles et al., 2001), *Toxoplasma* (Hay et al., 1983), and *Leptospira* (Salt and Little, 1977) have all been reported in UK wild rodent populations, and thus released beavers may become exposed. Ideally, wild rodents in the vicinity of the release sites should also be trapped and screened. There was no pathologic evidence of these pathogens on postmortem examination of the beavers. However, this status may change because of exposure to other animals. For example, *Giardia* genotypes A and B are considered zoonotic and present in other animals. In the United Kingdom *Giardia* spp. are present in livestock (Hutchison et al., 2004), dogs (*Canis lupus familiaris*; Batchelor et al., 2008), and water supplies (Smith et al., 2006).

In the fourth step criteria for release or exclusion of animals for reintroduction should be considered. If animals are positive for enteric pathogens (*Salmonella* spp., *Campylobacter* spp., *Clostridium* spp., *Yersinia* spp., and *Giardia* spp.), they would have to be treated and found negative prior to release or be excluded. *Leptospira* spp. were included in the screening protocols to monitor and compare prerelease versus postrelease and exposure in the field. Animals antibody positive for *Leptospira* spp. were not excluded from this trial, as wild rodents act as a reservoirs for various serovars such as *L. copenhageni*, *L. icterohaemorrhagiae*, and *L. saxkoebing*. *Leptospira autumnalis* has been isolated from water voles (*Arvicola amphibius*) and *L. javanica* from European hedgehogs (*Erinaceus europaeus*; Salt and Little, 1977). Eurasian beaver were found to have antibody to serovar ictero (*L. copenhageni* or *L. icterohaemorrhagiae*; Hartskeerl and Terpstra, 1996). No clinical disease or pathology was noted in relation to *Leptospira* infection in the Scottish Beaver Trial.

There are recurring reports of the detection of the beaver cercal fluke *Stichorchis subtriquetrus* in European wild beaver populations (Koubkova et al., 2002; Drodz et al., 2004; Sager et al., 2005). This parasite may go undetected in feces if burdens are low and if simple flotation techniques are used instead of sedimentation. *Stichorchis subtriquetrus* was detected in eight beavers: five from fecal samples and three on postmortem examination. The animals were not treated for this parasite because it is not deemed pathogenic under normal circumstances (Nolet et al., 1997). The fluke is specific to beavers (Vengust et al., 2009) and *S. subtriquetrus* is highly prevalent in beaver populations (Ahlen, 2001; Campbell-Palmer et al., unpubl.). Based on this information and on the absence of effective prophylactic treatment options, the beavers were reintroduced without treatment for this parasite.

The highest mortalities occurred during the rabies quarantine period. Infectious disease was a significant cause of death, as seen on necropsy of beavers in a Dutch translocation program (Nolet et al., 1997). Eighteen percent died from yersiniosis and 14% from leptospirosis. The pathogens were not isolated or considered contributing factors to the deaths of beavers in quarantine or following release. Results in necropsies of 60 wild beavers in Austria and Germany found that infectious disease was the second most common cause for beaver mortality (Steinbeck and Sieber, 2003).

The highest mortality occurred during quarantine, with the stress of captivity as a potential exacerbating factor. Because of Norway's rabies-free status (Office International des Epizooties [OIE], 2011) a request was made for alternative rabies quarantine import regulations. In 2010 the Scottish government allowed importation of subsequent animals without the 6-mo rabies quarantine requirement, provided they were accompanied by certification from the Norwegian state veterinarians that animals were from a rabies-free area and had undergone 1-mo quarantine in

Norway prior to export to the United Kingdom. Three animals were successfully introduced to Knapdale under these new regulations.

There is often a debate between those who prefer minimum intervention and those who prefer more hands-on monitoring of animals involved in reintroduction programs (Seddon, 1999). A balance needs to be reached between the potential stress and injury caused by repeated trapping of animals and subclinical disease going undiagnosed. Mathews et al. (2006) suggested a minimum of one follow-up session postrelease to allow changes in parasite and potential pathogen prevalence to be assessed. As part of the ongoing 5-yr trial, after release, the beavers in the Scottish Beaver Trial are to be observed and monitored monthly and trapped at least yearly if no abnormalities are observed in the interim. When animals are trapped they are weighed, and fecal and blood samples are collected. These samples are submitted for bacterial and parasitic enteric pathogens and *Leptospira* testing, hematology, and biochemistry. Any ill or injured animal observed during the trial will be caught to be treated on site by a veterinarian or removed from the reintroduction program. Opinions may differ concerning the appropriateness of intervention in such cases, as some prefer to let nature take its course, whereas others feel they have a duty to intervene, at least during the initial stages of a reintroduction program.

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