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# Evidence of *Leptospira* species and their significance during reintroduction of Eurasian beavers (*Castor fiber*) to Great Britain

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## Abstract

The Scottish Beaver Trial (SBT) reintroduced the Eurasian beaver (*Castor fiber*) in 2009 using wild-caught Norwegian beavers. This included a six-month prerelease quarantine in Devon, England. The International Union for Conservation of Nature (IUCN) and government guidelines for health screening were followed, including testing for *Leptospira* species. Unlicensed beavers, from Germany, were also identified in Scotland (Tayside) and Devon (later forming the River Otter Beaver Trial (ROBT)) and were health-screened under licence. Due to positive *Leptospira* species results and lack of prerelease screening in ROBT and Tayside, beavers from Germany and Norway (range sources) were screened. One hundred and fifty-six samples from 151 beavers were analysed by *Leptospira* species quantitative PCR (qPCR) (n=73 kidney (postmortem)/urine samples (antemortem)) or microscopic agglutination test (MAT, *Leptospira* pools 1–6) (n=83 serum samples). No beavers from Norway (95 per cent confidence interval (CI) 0–5.6 per cent, n=52), Tayside or SBT postrelease (95 per cent CI 0–4.6 per cent, n=63) tested positive. Seven beavers from Germany and Devon were positive. This gives an overall 9.3 per cent (95 per cent CI 5.2–15.1 per cent) exposure level, of which 4.6 per cent (95 per cent CI 1.9–9.3 per cent) suggested infection on a positive qPCR (n=1) or MAT titre of at least 1/400 (n=6), although none had abnormal physical, biochemical or haematological changes. This study suggests that *Leptospira* species infection in wild Eurasian beavers occurs at a low level, has no sex bias and does not appear to cause significant morbidity or mortality.

## Introduction

Leptospirosis is potentially a significant cause of morbidity and mortality in mammals, a zoonosis, and is particularly associated with rodents and mammals living in an aquatic, or semiaquatic, habitat.<sup>1–3</sup> Nomenclature for *Leptospira* species is somewhat complicated.

Most pathogenic leptospires are viewed as serovars of *Leptospira interrogans*, although cross-reactivity between other species is reported.<sup>4</sup> The nomenclature for the serovar name is that it should be written with an initial capital letter and should not be italicised, for example *L interrogans* serovar Icterohaemorrhagiae.<sup>4</sup> Rodents are considered important reservoir hosts and vectors for this pathogen, with the organism residing in the host's kidneys and spread via urine, entering the next host's body via mucocutaneous surfaces and wounds.<sup>3 5 6</sup> Leptospirosis can result in morbidity and mortality in some rodents, which may have a significant impact on a reintroduction's success.<sup>1 7</sup> It has been suggested that releasing immunologically naïve animals may increase the risk of postrelease morbidity and mortality, bringing into question the interpretation of prerelease health screening.<sup>1</sup> In addition to a species bias, a sex bias in many infectious diseases has been reported, with male rodents more likely to be infected, associated with interspecific and intraspecific fighting and greater spatial movement.<sup>8</sup>

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The Eurasian beaver (*Castor fiber*) has recovered from near extinction in the 19th century caused by persecution.<sup>9</sup> Protection measures and reintroduction programmes have ensured that this species is no longer of conservation concern.<sup>10</sup> Beavers have been the subject of several reintroduction projects throughout their former range across Europe, most recently in Scotland, UK.<sup>11 12</sup> The Scottish Beaver Trial (SBT) carried out in Knapdale, Argyll, Scotland from 2010 to 2015 involved the legal importation and release of wild-caught Norwegian beavers, subject to formal reintroduction best-practice protocols, including statutory quarantine; prerelease and postrelease health screening; and ongoing monitoring, including assessment of health, behaviours, reproduction and mortality rates.<sup>13-15</sup> Outside of this official process, a significant population (~114 active territories) of free-ranging beavers now exist throughout the Rivers Tay and Earn catchments in Perthshire, east Scotland (referred to subsequently as Tayside beavers), resulting from accidental escapees or alleged deliberate unlicensed releases.<sup>16</sup> Trapping and health screening of live beavers from Tayside as well as analysis of dead animals have demonstrated their genetic origins are from Bavaria, Germany.<sup>17</sup> Similarly a small family group of unlicensed beavers were identified on the River Otter, Devon (now the River Otter Beaver Trial, ROBT) whose genetic findings were identified as from Bavaria, Germany (R Campbell-Palmer, personal communication).

This study aimed to examine the incidence of *Leptospira* species exposure of the Eurasian beaver in Britain pre-reintroduction and post-reintroduction, including an assessment of the source population for the SBT (Telemark, Norway), Tayside and ROBT beavers (Bavaria, Germany), and a search for any association between positive *Leptospira* species results, sex bias, or ill-health detectable through physical and clinical testing.

## Materials and methods

### Leptospiral testing methods

A serological multiple serovar microscopic agglutination test (MAT) was used on serum samples from 65 individual Eurasian beavers as previously described.<sup>18</sup> Some beavers were resampled in subsequent years as part of the SBT, leading to a total of 83 samples tested by *Leptospira* species MAT. MAT tests for all six pools of *Leptospira* species currently identified and was carried out by the Animal and Plant Health Agency, UK Government Laboratories in Weybridge, Surrey, UK. It is currently recognised as the reference test method for the serodiagnosis of leptospirosis both in human beings and animals.<sup>19 20</sup> Serologically positive samples were then further analysed by MAT to determine the antibody titre to an individual *Leptospira* serovar. Positive results were interpreted as any titre greater than 1:100, with titres of at least 1:400 being typically associated with

active/recent infections.<sup>20</sup> All beavers sampled by MAT serology were residents in Britain at the time of sampling.

A real-time quantitative PCR (qPCR) test was used on kidney tissue samples from 65 Eurasian beavers for *Leptospira* species DNA.<sup>21</sup> These animals were either submitted for postmortem from wild British beavers that had been shot or involved in motorised vehicle collisions, or as tissue samples from wild beavers in the Telemark region of Norway and from Bavaria, Germany that had been legally dispatched by wildlife staff. Samples were frozen within one to four hours of collection at -80°C until testing was performed. The same qPCR test was used to test urine samples from eight Eurasian beavers from Scotland, collected either at the time of postmortem or from live beavers undergoing health screening testing as a free-catch sample involuntarily voided.

### General health screening methods

Live beavers were trapped using Bavarian beaver traps. They were transferred to burlap sacks, and via a face mask administered isoflurane in 100 per cent oxygen to induce general anaesthesia and then intubated and maintained on isoflurane while samples were taken. Blood samples were taken from the ventral midline tail-vein plexus, approximately halfway down the length of the tail.

All beavers that were blood-sampled for leptospiral antibody testing had full serum biochemical and haematology assessment, which was compared with previously published ranges.<sup>22</sup> Serum biochemical analysis included measuring blood urea nitrogen, creatinine, total calcium, total phosphorus, total proteins, albumin, globulins, alanine transferase, total bilirubin, bile acids, amylase, sodium, potassium and glucose. Haematology assessment included packed cell volume, haemoglobin concentration, leucocyte count and differential, erythrocyte count, and blood smear cytology assessment. A physical examination for signs of ill-health was also performed. All Tayside beavers that received a postmortem examination had kidney samples fixed in 10 per cent neutral buffered formalin for seven to 10 days, then processed to paraffin wax blocks. Two 4-µm thick sections were cut and stained with haematoxylin and eosin for histopathological examination.

### Population of beavers studied

#### Scottish Beaver Trial

These beavers originated from the wild in the Telemark region of Norway. They were quarantined for a period of one month in Norway before being imported to the UK. The initial animals then underwent six-month rabies quarantine in Devon, England before being released as part of SBT in Knapdale, Argyll, Scotland. During the SBT and as part of the Scottish Government

licence, the beavers were recaptured annually where possible. Testing included a blood sample and physical examination to assess their health status as described above and serological MAT for six pools of *Leptospira* species. Urine samples, where obtained, were tested as described above by qPCR for *Leptospira* species DNA.

#### Tayside beavers

These beavers have been illegally released or escaped from captivity in the east of Scotland along the Rivers Tay and Earn and now thought to number more than 400 individuals.<sup>16</sup> Tissue sampling was carried out on animals killed through legal lethal control methods by local landowners, animal predation or motorised vehicle collisions.

Blood sampling and urine collection of live animals were carried out as part of a Scottish Government-licensed project to assess the health status and presence/absence of zoonotic disease. Blood samples from live animals were tested serologically by MAT for six pools of *Leptospira* species, as well as for haematology and serum biochemistry. Urine and tissue (kidney) samples, where obtained, were tested as described above by qPCR for *Leptospira* species DNA.

#### Telemark, Norway (source for SBT)

These were wild beavers that either formed part of the original population used for the SBT or that were shot legally during the Norwegian hunting season within the catchment areas that supplied the SBT beavers. Kidney samples were tested as described above by qPCR for *Leptospira* species DNA.

#### Bavaria, Germany (source for Tayside and ROBT)

These were wild beavers shot legally by wildlife staff as part of German beaver management programmes, or where indicated Bavarian captive-bred animals subsequently moved to Scotland, UK. Kidney samples were tested as described above by qPCR for *Leptospira* species DNA. Blood samples from live animals were tested serologically by MAT for six pools of *Leptospira* species serovars, as well as for haematology and serum biochemistry.

#### River Otter Beaver Trial

These had presumably escaped from captivity and established themselves on the River Otter, Devon, England. As part of a Defra-licensed health screening programme in conjunction with the Devon Wildlife Trust, these beavers were caught for health screening. Blood sampling was repeated a year later to assess their ongoing health status. These were tested serologically by MAT for six pools of *Leptospira* species serovars, as well as for haematology and serum biochemistry. These beavers have been included in this study to help explain some of the results from SBT beavers during their quarantine in Devon.

#### Statistical analysis

Confidence interval (CI) analysis of proportions was performed using Minitab 17 (Minitab, Pennsylvania) at a 95 per cent level of confidence.

## Results

### SBT beavers

Results are detailed in [table 1](#).

Period of time	Beavers testing positive by MAT serology (95% CI)	Beavers testing positive by urine qPCR for <i>Leptospira</i> DNA (95% CI)	Beavers demonstrating haematology or serum biochemical results outwith published ranges <sup>22</sup> (95% CI)	Serovars of <i>Leptospira</i> species identified/ number of beavers positive (titres seen)	Sex of beavers tested male:female (sex of beavers testing positive male/female)
Prerelease	30%/9 of 30 beavers (14.7%–49.4%)	0%/0 of 1 beaver (0%–95%)	0%/0 of 31 beavers (0%–9.5%)	Pool 1 serovars: Canicola/6 beavers (titre of 1/100 in 5 beavers, 1/400 in 1 beaver) Copenhagi/9 beavers (titre of 1/100 in 2 beavers, 1/200 in 3 beavers, 1/400 in 1 beaver, 1/800 in 3 beavers) Icterohaemorrhagiae/4 beavers (titre of 1/200 in 3 beavers, 1/400 in 1 beaver) Pool 3 serovars: Autumnalis/1 beaver (titre of 1/200 in 1 beaver) Pool 5 serovars: Javanica/2 beavers (titre of 1/3200 in 1 beaver, 1/12,800 in 1 beaver)	14:17 (5:4)
One year postrelease	12.5%/1 of 8 beavers (3.2%–52.7%)	NA	0%/0 of 8 beavers (0%–31.2%)	Icterohaemorrhagiae/1 beaver (titre of 1/200)	5:3 (1:0)
Two years postrelease	0%/0 of 4 beavers (0%–52.7%)	NA	0%/0 of 4 beavers (0%–52.7%)	NA	1:3 (0:0)
Three years postrelease	0%/0 of 3 beavers (0–63.2%)	NA	0%/0 of 3 beavers (0–63.2%)	NA	1:2 (0:0)
Four years or more postrelease	0%/0 of 2 beavers (0%–77.6%)	NA	0%/0 of 2 beavers (0%–77.6%)	NA	0:2 (0:0)

MAT, microscopic agglutination test; NA, not applicable; qPCR, quantitative PCR; SBT, Scottish Beaver Trial.

Prerelease seropositivity was 30 per cent (95 per cent CI 14.7–49.4 per cent, n=30), with pool 1 serovars *Canicola* (six beavers), *Copenhagi* (eight beavers) and *Icterohaemorrhagiae* (four beavers); pool 3 serovar *Autumnalis* (one beaver); and pool 5 serovar *Javanica* (one beaver) being identified. Although all originated from the wild in Norway, this prerelease testing coincided with the end of a six-month period of quarantine in Devon. Of these results, titres of at least 1/400 (cut-off point assumed to be associated with active infection) were only seen for the following serovars: *Icterohaemorrhagiae*, *Copenhagi* and *Javanica*, with *Javanica* producing the highest titres in two beavers (1/12,800 and 1/3200).<sup>20</sup> Prerelease qPCR was available from urine of one individual which was negative.

Postrelease data are detailed in table 1. Positive results postrelease were identified in one beaver (serovar *Icterohaemorrhagiae*) in an individual that had a positive titre before release. The titre in this individual remained the same as its prerelease titre (1/200) to *Icterohaemorrhagiae*. This individual animal also tested positive to serovars *Copenhagi* (1/400), *Canicola* (1/100) and *Javanica* (1/3200), prerelease, but on retesting a year after release into Knapdale as part of the SBT it was serologically negative to these serovars. Out of nine beavers testing positive on serology prerelease, three were not retrapped. Six beavers that were seropositive before release became seronegative (five tested negative by MAT at one year; two tested negative at two years; and two tested negative at three years postrelease). No beavers seroconverted to *Leptospira* species postrelease and no sex bias was noted in those showing exposure to *Leptospira* species versus those that did not.

All blood biochemistry and haematology values of SBT beavers prerelease and postrelease were within published normal parameters.<sup>22</sup> No beavers demonstrated evidence of ill-health on clinical examination under anaesthesia.

### Tayside beavers

Results are detailed in table 2. Forty samples were tested from 35 beavers (five beavers had both MAT serology and urine qPCR testing). No beavers tested positive on qPCR of urine or kidney (n=15) or MAT serology (n=25). No evidence of nephritis typical of active infection by *Leptospira* species was determined on histopathology

(n=8). All blood biochemistry and haematology values were within published normal parameters.<sup>22</sup> No beavers demonstrated evidence of ill-health on clinical examination. No sex bias was evident in those beavers demonstrating exposure to *Leptospira* species versus those that did not.

### Norway beavers

Results are detailed in table 3. No evidence of *Leptospira* species DNA was detected in any sample by qPCR (n=52).

### Bavarian, Southern Germany beavers

Results are detailed in table 4. One beaver tested positive to *Leptospira* species DNA on qPCR testing of the kidney. One beaver tested positive on MAT serology testing, and further analysis demonstrated it seroconverted to a total of six serovars (*Icterohaemorrhagiae*, *Copenhagi*, *Canicola*, *Bataviae*, *Javanica* and *Zanoni*), with only *Icterohaemorrhagiae* having a titre of at least 1/400 suggestive of active/recent infection.<sup>20</sup> Therefore a total of two beavers out of nine tested (22.2 per cent, 95 per cent CI 28.1–60 per cent) were positive for *Leptospira* species. No sex bias was noted in those showing exposure to *Leptospira* species versus those that did not.

### ROBT beavers

Results are detailed in table 5. In year 1 of the study, one beaver tested positive on MAT (25 per cent of those tested) to serovar *Javanica* at a titre of 1/800—a serovar seen during the postimport quarantine of the SBT beavers in Devon and a titre level suggesting active/recent infection.<sup>20</sup> On resampling two beavers tested positive on MAT serology (100 per cent of those tested), but these were to three novel serovars: *Australis* and *Bratislava* in one beaver and *Hardjoprajtino* in the other. No sex bias was noted in those showing exposure to *Leptospira* species versus those that did not. All blood biochemistry and haematology values were within published normal parameters.<sup>22</sup> No beavers demonstrated evidence of ill-health on clinical examination.

### Serovars of *Leptospira* detected

Table 6 shows the incidence of the *Leptospira* species serovars identified on MAT serological testing, with the

Beavers testing positive by MAT serology/number of beavers tested	Beavers demonstrating haematology or serum biochemical results outwith published ranges <sup>22</sup> (95% CI)	Beavers testing positive by kidney or urine qPCR for <i>Leptospira</i> DNA (95% CI)	Serovars of <i>Leptospira</i> species identified/number of beavers positive	Beavers showing signs of renal histopathological changes/beavers with no renal histopathological changes (95% CI)	Sex of beavers tested male:female:unidentified (sex of beavers testing positive male:female:unidentified)
0/25	0%/0 of 25 beavers (0%–11.3%)	0%/0 of 15 beavers (0%–18.1%)	NA	0%/0 of 9 beavers (0%–28.3%)	19:14:2 (0:0:0)

MAT, microscopic agglutination test; NA, not applicable; qPCR, quantitative PCR.

**Table 3** Norwegian free-ranging beavers tested: results of qPCR for *Leptospira* species DNA

Beavers testing positive by kidney or urine qPCR	(95% CI)
0%/0 of 52 beavers	(0%–5.6%)
qPCR, quantitative PCR.	

most commonly seen serovar being Copenhageni with 10 beavers demonstrating an immunological response, followed by Canicola with seven, Icterohaemorrhagiae with six and Javanica with four beavers. The rest of the serovars identified (Autumnalis, Australis, Bataviae, Bratislava, Prajitno and Zanoni) were each only reported in one individual beaver. From this it can be seen that some of the serologically positive beavers were positive to multiple leptospire. The total number of antibody positive beavers in this study was 14, and of these only five individuals were positive to just one serovar of leptospire. Of the other nine beavers, four were positive to two serovars of leptospire; one was positive to three serovars of leptospire; one was positive to four serovars of leptospire; and one was positive to five serovars of leptospire. The greatest titres seen were to serovar Javanica, with two beavers in quarantine for the SBT showing titres of 1/3200 and 1/12,800, respectively.

### Overall results summary

Some of the *Leptospira* species qPCR and MAT were taken from the same individual, meaning out of 156 tests (83 MAT and 73 qPCR) a total of 151 individuals were actually tested. Of these, 14 produced a positive result, 9.3 per cent (95 per cent CI 5.2–15.1 per cent) of the population. Of the 14 positive tests, one qPCR test demonstrated current infection and six MAT results (based on titres  $\geq 1/400$ ) suggested active/recent infection, meaning the actual number of beavers that could be construed as being infected/recently infected at the time of testing was 7 of 151 beavers (4.6 per cent, 95 per cent CI 1.9–9.3 per cent).<sup>20</sup> The most

**Table 4** Bavarian beavers tested: results of MAT with identified serovars, qPCR for *Leptospira* species DNA and sex ratios of tested animals

Beavers testing positive by MAT serology (95% CI)	Beavers testing positive by kidney or urine qPCR (95% CI)	Percentage overall testing positive to <i>Leptospira</i> species (95% CI)	Serovars of <i>Leptospira</i> species identified/ number of beavers positive (titres seen)	Sex of beavers tested male:female (sex of beavers testing positive male:female)
25%/1 of 4 beavers (0.6%–80.6%)	20%/1 of 5 beavers (0.5%–71.6%)	22.2%/2 of 9 beavers (28.1%–60%)	Pool 1 serovars: Canicola/1 beaver (titre of 1/200) Copenhageni/1 beaver (titre of 1/200) Icterohaemorrhagiae/1 beaver (titre of 1/400) Pool 5 serovars: Bataviae/1 beaver (titre of 1/200) Javanica/1 beaver (titre of 1/200) Zanoni/1 beaver (titre of 1/200)	4:5 (1:1)
MAT, microscopic agglutination test; qPCR, quantitative PCR.				

**Table 5** ROBT beavers tested in first-year and second-year surveys: results of MAT with identified serovars, haematology and biochemistry testing, and sex ratios of tested animals

Period of time	Beavers demonstrating haematology or serum biochemical results outwith published ranges <sup>22</sup> (95% CI)	Beavers testing positive by MAT serology (95% CI)	Serovars of <i>Leptospira</i> species identified/ number beavers positive (titres seen)	Sex of beavers tested male:female (sex of beavers testing positive male:female)
First-year results	0%/0 of 4 beavers (0%–52.7%)	25%/1 of 4 beavers (0.6%–80.6%)	Pool 5: Javanica/1 beaver (1/800)	2:2 (1:0)
Second-year results	0%/0 of 2 beavers (0%–77.6%)	100%/2 of 2 beavers (22.4%–100%)	Pool 3: Australis/1 beaver (titre of 1/800) Bratislava/1 beaver (titre of 1/100) Pool 6: Prajitno/1 beaver (titre of 1/100)	1:1 (1:1)
MAT, microscopic agglutination test; ROBT, River Otter Beaver Trial.				

commonly seen *Leptospira* serovar was Copenhageni, with the greatest titres being seen against Javanica, both associated with the quarantine period of the SBT beavers based in Devon.

### Discussion

Mortalities due to leptospirosis have been implicated in contributing to the failure of one Eurasian beaver reintroduction project in the Netherlands.<sup>7</sup> *L. interrogans* serovar Icterohaemorrhagiae infection was diagnosed in one of these deaths with a titre in excess of 1/20,000. In Switzerland, 13 beavers were identified as having died of leptospirosis, and the serovars included predominantly Icterohaemorrhagiae (four beavers), as well as Autumnalis (two beavers), Australis (one beaver) and Sejroe (two beavers).<sup>23</sup> As a result, some authors have suggested avoiding the release of immunologically naïve animals into an environment where exposure to *Leptospira* species is likely.<sup>1</sup> The reservoir host for serovar Icterohaemorrhagiae is believed to be the brown rat (*Rattus norvegicus*).

In this study, four beavers tested serologically positive for serovar Icterohaemorrhagiae while in quarantine in Devon, England, and a further beaver tested serologically positive from Bavaria, Germany; two of these five animals had titres of at least 1/400, indicating recent exposure/infection.<sup>20</sup> None of these beavers showed signs of clinical disease, nor did they show elevated renal parameters or leucocyte counts suggestive of kidney disease or an immunological response. This suggests that the Eurasian beaver can be exposed to serovar Icterohaemorrhagiae without fatal results and would support the view that other disease factors were at play in previously reported mortalities.<sup>7,23</sup> Other significant causes of infectious disease that were likely to have impacted negatively on the Netherlands' reintroduction included coinfection with *Yersinia*

**Table 6** Prevalence of *Leptospira* species serovars identified by MAT serological testing in 14 positive Eurasian beavers and their study population (SBT; ROBT; Bavaria, Germany (Bavarian))

<i>Leptospira</i> species serovars	Number of beavers with a titre $\geq 1/100$	Number of beavers and their study population(s)	Highest reported antibody titre
Australis	1	1 ROBT	1/800
Autumnalis	1	1 quarantine Devon, SBT (prerelease)	1/200
Bratislava	1	1 ROBT	1/400
Bataviae	1	1 Bavaria, Germany	1/200
Canicola	7	6 quarantine Devon, SBT (prerelease) 1 Bavaria, Germany	1/400 (SBT quarantine)
Copenhagi	10	9 quarantine Devon, SBT (prerelease) 1 Bavaria, Germany	1/800 (SBT quarantine)
Icterohaemorrhagiae	6	4 quarantine Devon, SBT (prerelease) 1 SBT year 1 (previously positive animal during quarantine, Devon) 1 Bavaria, Germany	1/400 (SBT quarantine)
Javanica	4	1 Bavaria, Germany 1 ROBT 2 quarantine Devon, SBT (prerelease)	1/12,800 (SBT quarantine)
Prajitno	1	1 ROBT	1/100 (ROBT)
Zanoni	1	1 Bavaria, Germany	1/200 (Bavaria, Germany)

MAT, microscopic agglutination test; ROBT, River Otter Beaver Trial; SBT, Scottish Beaver Trial.

species, and this combined with the physiological stress of translocation may have suppressed the immune system response, resulting in leptospirosis-associated deaths, a finding supported by similar situations in North American beavers (*Castor canadensis*) where mortalities have occurred.<sup>7 24 25</sup>

In this study, serovar Hardjo-prajitno seroconversion was seen in one animal from the ROBT (titre of 1/100). ROBT has areas where cattle have access to the river (uncommon in other areas looked at in this study), which may explain exposure as the host species for Hardjo-prajitno is thought to be domestic cattle.<sup>26</sup> The low titre demonstrated did not support active/recent infection, and renal biochemical parameters and haematological values were within normal ranges.<sup>20 22</sup>

There was no evidence of leptospirosis in beavers in the Telemark region of Norway. Previous studies in this geographical area and Scandinavia have suggested leptospirosis is present in wild canids, with high levels in red foxes (*Vulpes vulpes*) (59.6 per cent) and wolves (*Canis lupus*) (67.7 per cent) due to serovars of leptospire such as Canicola and Icterohaemorrhagiae.<sup>27</sup> It may be that due to their semiaquatic nature, contact between Eurasian beavers and these canids is uncommon, and the red fox is unlikely to be able to successfully predate beavers, further reducing the likelihood of contact. In the authors' opinion this also made it more likely that those Norwegian beavers imported for SBT that were seropositive for *Leptospira* species became so during the period of quarantine in Devon, rather than before leaving Norway, suggesting possible rodent exposure may have been an issue at this time.

Of greater importance to the SBT was the absence of evidence of *Leptospira* species in beavers in Scotland. The only serologically positive animals were those whose titres were positive before movement here after a period of isolation/quarantine in Devon, England. Once released into SBT, all showed negative titres at

the annual health assessment, except one individual that nonetheless showed a significantly reduced titre to one serovar (Icterohaemorrhagiae) to which it was previously positive. No beaver released into SBT showed any signs of seroconversion to new *Leptospira* serovars. Tayside beavers similarly were all negative serologically and on qPCR testing, suggesting no evidence of reservoir/carrier status.

In Bavarian beavers some serological evidence of *Leptospira* species exposure was demonstrated (n=1) and one was positive on qPCR testing, giving an overall incidence of 22.2 per cent (95 per cent CI 28.1–60 per cent, n=2/9), although the number tested was small and this may have skewed the results. Similarly, ROBT beavers showed an overall incidence of 50 per cent (95 per cent CI 11.8–88.1 per cent, n=3/6). The numbers tested were small, but this does fit with the positive serological results obtained in nine of the original 30 beavers (30 per cent) quarantined for the SBT in Devon at the start of the trial and suggests a higher exposure to leptospire in this region than in Scotland. The evidence of beavers being exposed to leptospire and likely becoming infected followed by the same animals showing subsequent waning or negative titres once part of the SBT release with no evidence of clinical disease or mortalities is at odds with publications, suggesting that beavers die rapidly after *Leptospira* species infection.<sup>23</sup>

Findings in this study support the suggestion that beavers are aberrant hosts, as the incidence overall is very low at 9.3 per cent of all beavers tested, of which 4.6 per cent could be considered as infected at or around the time of sampling due to either positive qPCR or MAT titres of at least 1/400.<sup>28</sup> This contradicts publications that suggest that beavers behave similarly to other rodents that live close to water (eg, brown rat), where continuous infection and shedding of the bacterium into an environment that favours its persistence are commonplace.<sup>29</sup> Additional

work suggests that a semiaquatic lifestyle is not closely associated with *Leptospira* species infection in rodents, but synurbanisation (adaption of wildlife populations to urban environments) is a significant factor.<sup>30</sup> Further work in Canada demonstrated the incidence of leptospirosis-positive animals increased with areas of higher human density and where domestic livestock was abundant.<sup>31</sup> The populations in this study that were predominantly negative (Norwegian, SBT and Tayside populations) were not closely associated with urban areas, being largely rural and mainly remote. The populations with some level of exposure and potential infection (Devon and Bavaria) were more closely associated with human habitations. The ROBT beavers inhabit a river that runs through an area regularly used for human recreation as well as being close to fields of domestic dairy cattle. The Bavarian beavers sampled were all from an area closely associated with Munich and its suburbs. Further supporting this theory, in this study the serovars of *Leptospira* most commonly seen in these areas are species associated with other reservoir hosts found in populated areas such as brown rats (*Icterohaemorrhagiae* and *Copenhagi*) and domestic dogs (*Canicola*). Less commonly reported serovars such as *Hardjo-prajitno* seen in the ROBT beavers have domestic cattle as their reservoir host and would fit with the landscape where dairy cattle farming is commonplace.<sup>26</sup> Other serovars of *Leptospira* seen in this study are more loosely connected to a reservoir host such as *Autumnalis*, *Australis*, *Bataviae* and *Zanoni*, which have all been associated with domestic cattle, horses and pigs (although *Autumnalis* is also associated with mice, *Mus musculus*); *Bratislava*, which has been associated with pigs, horses, hedgehogs and domestic dogs; and *Javanica*, which has been associated with brown rats and domestic cattle.<sup>32</sup> Serovars *Australis* (one from the ROBT), *Autumnalis* (one from Devon quarantine of SBT) and *Canicola* (six from Devon quarantine of SBT and one from Bavaria, Germany) have all been reported on serological testing of North American beavers in San Pedro River Basin, Sonora, Mexico at low titres and thought to be associated with exposure to domestic animals that act as source/reservoir hosts.<sup>25</sup> In other studies of rodents in Europe, in addition to the association between the brown rat and serovar *Icterohaemorrhagiae*, other *Leptospira* serovars assumed to be associated with rodents as reservoir hosts have included *Kirschneri*, *Interrogans* and *Borgpetersenii*, with rodents of the genus *Microtus* species being most commonly associated with infection (13 per cent of those tested in one study).<sup>33 34</sup> One study associated *L. interrogans* with Eurasian beaver mortalities in Germany which contains serovars such as *Canicola*, *Icterohaemorrhagiae* and *Australis*, all of which were seen in this study without mortalities.<sup>29</sup> There was no

significant association in this study with sex as has been previously shown for other rodent pathogens.<sup>8</sup>

Another factor identified in the environmental risk of leptospirosis exposure is the soil type favouring survival of the organism, with sabulous loamy clay soils, as seen in Devon ROBT areas, significantly increasing the risk.<sup>35</sup> Soil types in Tayside and SBT areas are acidic, volcanic soils, making survival of leptospires in the environment less likely.

Kidney qPCR testing is only suitable for postmortem diagnosis of infection and so has limited use in the live animal as a screening technique. Urine samples were tested by qPCR, but these were small in number as it is technically difficult to collect urine as the urinary tract in both sexes exits inside a cloaca, making it hidden from external view. This makes demonstrating a non-shedding state in an individual difficult. MAT serological testing does allow detection of exposure and possible diagnosis of active/recent infection based on titre results. Interpreting positive serological MAT results relative to the health of the animal and its suitability for release is complicated, and positive, even high titres do not always equate to clinically detectable disease or reduced survival rates as shown here. Mortalities and leptospirosis in the Eurasian beaver are more likely to be associated where immunosuppressive conditions (eg, physiological stress) or concurrent infections with other pathogens (eg, *Yersinia* species) are present. The Eurasian beaver can clearly, as with any mammal, become infected with *Leptospira* species. However, identifying it as a reservoir host responsible for long-term or even short-term shedding of the bacterium based on current evidence seems unlikely.

### Summary

The large number of kidney tissue samples tested by qPCR that were all negative suggests beavers are not commonly persistently infected with *Leptospira* species in Scotland and Norway, and so are unlikely to be a significant reservoir host for *Leptospira* species in these areas. The evidence of positive serological responses to serovars of *Leptospira* that have previously been reported to cause mortalities in beavers without clinical signs of disease suggests such mortalities may have had concurrent or other immunosuppressive disease. Seroconversion and infection may be more likely in areas close to human habitation/increased urbanisation, and the incidence increases in areas that have greater levels of *Leptospira* species reservoir hosts in close proximity to their habitat as well as being associated with loamy clay soils favouring survival of the organism in the environment.<sup>30</sup> This highlights the importance of taking multiple factors into account when considering reintroduction of a species and suggests that Eurasian beavers acting as a *Leptospira* species reservoir host is an uncommon event.



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