

## Short Communication

1 **Investigating infectious disease threats to the recovery of the European polecat in**2 **Britain**

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21 **Abstract**

22 The European polecat (*Mustela putorius*) almost became extinct in Britain in the early 20<sup>th</sup>  
23 century, but populations are now recovering. As seen in other endangered carnivore  
24 populations, disease is one potential threat to recovery. This study assessed exposure of wild  
25 polecats (n=149) to three, multi-host pathogens which could limit reproduction and/or cause  
26 morbidity and mortality. Serum, lung and brain samples were collected from polecats which  
27 died from 2011 to 2016 across Britain. Exposure to *Toxoplasma gondii* and 12 *Leptospira*  
28 serovars was assessed serologically by antibody detection using the latex agglutination test  
29 and microscopic agglutination test respectively, and the presence of Canine Distemper Virus  
30 (CDV) RNA in lung and brain tissue samples was assessed using PCR. Generalised linear  
31 models were used to test for relationships between exposure to each pathogen and season,  
32 sex, age, and location.

33

34 All organ samples tested PCR negative for CDV (95% CI 0.00%-0.05%). There was evidence  
35 of frequent exposure to *T. gondii* with a recorded seroprevalence of 71.8% (95% CI 64.2%-  
36 79.4%) and moderate exposure to *Leptospira* serovars, 14.5% (95% CI 8.6%-20.4%). Season,  
37 sex, age, and location were not significantly associated with exposure to *T. gondii* or  
38 *Leptospira* serovars.

39

40 Evidence of exposure to *T. gondii* and *Leptospira* serovars in European polecats could  
41 potentially affect mortality, longevity or fecundity. Further studies are warranted to assess the  
42 impact of these pathogens on polecat populations in Britain.

43

44 **Keywords:** *Canine distemper virus, Leptospira, Mustela putorius, Toxoplasma gondii.*

45

46 During the 17th and 18th centuries, the European polecat (*Mustela putorius*) was a common  
47 species with a widespread distribution throughout Britain (Langley and Yalden, 1977). From  
48 1800, their numbers started to decline, partly due to habitat change but also as a result of  
49 intensive predator control by game-keepers (Langley and Yalden, 1977). By 1850, polecats  
50 were almost extinct from the border counties of Scotland and were very rare in England's  
51 south-eastern counties (Langley and Yalden, 1977). The population reached a nadir by 1915  
52 and essentially existed in a refugium within a 40-mile radius around Aberystwyth, Wales  
53 (Langley and Yalden, 1977). As a result of reduced persecution pressures during the early  
54 20<sup>th</sup> century, the polecat population started to recover through natural recolonisation and by  
55 1968 polecats were reported to be present throughout Wales and the border counties (Langley  
56 and Yalden, 1977). National polecat distribution surveys started in the 1980s and by the 21<sup>st</sup>  
57 century there was an increased number of records in Derbyshire, Buckinghamshire,  
58 Berkshire, Wiltshire, Dorset and Hampshire (Birks, 2008; Croose, 2016). By 2015, polecats  
59 had recolonised Wales and most of central and southern England whilst unofficial releases  
60 meant that they had become established in Cumbria, Argyll and Perthshire (Birks, 2008;  
61 Croose, 2016). The British polecat population was last estimated at 83,300 (Mathews *et al.*,  
62 2018).

63

64 A number of factors may limit further recovery of polecat populations, including prey and  
65 habitat availability, predator controls, secondary exposure to anticoagulant rodenticides and  
66 infectious diseases (Sainsbury *et al.*, 2019). There are several examples of infectious diseases  
67 resulting in declines of other carnivore species (Johnson *et al.*, 2010; Soulsbury *et al.*, 2007).

68 In general, multi-host pathogens pose the greatest threat since they can be maintained in  
69 domestic and/or other wildlife populations and spill-over to affect susceptible species  
70 (Alexander *et al.*, 2010). As there is scant information on pathogen exposure and disease in  
71 polecats in Britain, this study focused on infectious, multi-host pathogens which polecats  
72 could be exposed to through their behaviour and dietary preferences: Canine Distemper Virus  
73 (CDV), *Toxoplasma gondii*, and *Leptospira* serovars.

74

75 The three pathogens were chosen because they can cause mortality, morbidity and reduced  
76 fecundity in related mustelid species and so may impact polecat population recovery. For  
77 example, the mortality rate of CDV infection in domestic ferrets (*Mustela furo*), thought to be  
78 genetic descendants of the wild polecat (Costa *et al.*, 2013; Sato *et al.*, 2003), is almost 100%  
79 (Kiupel and Perpiñán, 2014). Canine distemper virus caused the near elimination of remnant  
80 populations of wild black-footed ferrets (*Mustela nigripes*) (Thorne and Williams, 1988).  
81 Similarly, infection with *T. gondii* has resulted in high mortality rates in a captive colony of  
82 black-footed ferrets (Burns *et al.*, 2003). An outbreak of toxoplasmosis in farmed American  
83 mink (*Neovison vison*) has also resulted in abortion, and ataxia in kits (Frank, 2001), and  
84 farmed ferrets, suspected of being infected with *T. gondii*, have had symptoms of protracted  
85 anorexia and muscle spasms (Thornton and Cook, 1986). By extrapolating information from  
86 the domestic ferret, it is suspected that wild polecat populations could be negatively impacted  
87 by infection with *Leptospira* serovars through lower reproductive success and reduced  
88 individual health and longevity as a result of spontaneous abortions and renal damage  
89 (Swennes and Fox, 2014).

90

91 To obtain baseline data to inform future studies on the impacts of disease on polecat  
92 populations, this study aimed to quantify the exposure of British polecats to CDV, *T. gondii*  
93 and *Leptospira* serovars, and to assess host and environmental factors associated with  
94 exposure. Based on previous studies on mammals in Britain, it was hypothesised that polecats  
95 would be likely to have been exposed to *T. gondii* and *Leptospira* spp., but infection with  
96 CDV is not expected or may be present at a very low level.

97

98 One hundred and forty-nine polecat carcasses (32 females; 117 males. Table 1) were  
99 collected across most of the polecat range in Britain. Carcasses were submitted to the Vincent  
100 Wildlife Trust between 2011 and 2016, as part of a national species distribution survey, and  
101 tissue and blood sample collections were carried out. Samples of serum were collected by  
102 pipetting any blood visible within the body cavities, and lung and brain samples were  
103 collected where the condition of the carcass allowed (serum samples from n=131; tissue  
104 samples from n=79). Additional information was recorded including sex, the collection date  
105 and location. As part of a separate project, a subset of polecats (n=62) had their age estimated  
106 in months (Sainsbury *et al.*, 2018) by sectioning the canines and analysing the cementum  
107 layers microscopically (Matson *et al.*, 1993). This subset of polecats was subsequently  
108 categorised as adults or juveniles (further details in Supplemental Material 1).

109

110 Serum samples were screened for antibodies against *T. gondii* and a panel of 12 *Leptospira*  
111 serovars, and tissue samples were screened for infection with CDV by PCR. As blood  
112 samples were collected from polecat carcasses found opportunistically, there was a variable  
113 length of time from death until blood collection from the carcass. To ensure results were  
114 standardised, each serological assay included sample quality checks, filtering and assay

115 sensitivity testing (Supplemental Material 2). Screening of polecats in this study for CDV  
116 was carried out by PCR testing of tissues since the virus can be detected in lung and other  
117 organs from two days post-experimental infection (Kiupel and Perpiñán, 2014). In addition,  
118 haemolysis of some samples affected serological detection of CDV antibodies. Detection of  
119 exposure to *T. gondii* was by Latex Agglutination Test (LAT) (MAST Toxoreagent  
120 Toxoplasma Test, RST7001, Mast Group Ltd, Bootle, UK). An in-house *Leptospira*  
121 Microscopic Agglutination Test (LMAT) was used to test for exposure to *Leptospira*  
122 serovars. Further information on testing methodology is available in Supplemental Material  
123 2.

124  
125 To investigate associations between *T. gondii* and *Leptospira* serovar seroprevalence and host  
126 and environmental factors, the following explanatory variables were investigated: season,  
127 sex, and spatial location (latitude and longitude). A second model was run, with the subset of  
128 animals which had age estimated, including age along with the previous explanatory  
129 variables. A generalised linear model with binomially distributed errors and a logit link was  
130 used to test for associations between the explanatory variables and each pathogen (exposed,  
131 unexposed) separately. A maximal global model was fitted that included all explanatory  
132 variables and an interaction term between latitude and longitude. A backwards stepwise  
133 model selection was used, based on Akaike's information criterion (AIC). In order to choose  
134 the best model, variables were dropped sequentially by assessing the effects that their  
135 removal had on the model's AIC (Horton and Kleinman, 2015). The model with the lowest  
136 AIC value was selected. Statistical analyses were carried out in RStudio version 1.0.136 (R  
137 Development Core Team, Vienna, Austria).

138

139 The seroprevalence against *T. gondii* in polecats was 71.8% (n=94/131; 95% Confidence  
140 Interval (95% CI) = 64.2%-79.4%) (Table 2). The seroprevalence against *T. gondii* was  
141 61.2% (30/49; 95% CI = 46.2%-74.5%) for adults and 76.9% (10/13; 95% CI = 46.0%-  
142 93.8%) for juveniles, with exposure from two months old. The location of exposed and  
143 unexposed polecats is shown in Fig. 1. The overall *Leptospira* serovar seroprevalence was  
144 14.5% (19/131; 95% CI = 8.6%-20.4%) with exposure to three out of twelve *Leptospira*  
145 serovars tested: Bratislava 7.6% (10/131; 95% CI = 3.2%-12.0%); Saxkoebing 6.3% (8/127;  
146 95% CI = 1.9%-10.7%) and Icterohaemorrhagiae 1.5% (2/131; 95% CI = 0%-3.5%) (Table  
147 3). No exposure was detected to the other nine *Leptospira* serovars (Autumnalis, Canicola,  
148 Pomona, Ballum, Hardjo bovis, Tarasovi, Javanica, Altodouro, Grippytyphosa). Adult  
149 *Leptospira* serovar seroprevalence was 12.2% (6/49; 95% CI = 5.1%-25.5%) and 23.1%  
150 (3/13; 95% CI = 6.2%-54.0%) for juveniles, with exposure detected from four months old.  
151 The location of exposed and unexposed polecats is shown in Fig. 1. A total of 15 polecats  
152 tested seropositive for both *T. gondii* and *Leptospira* serovars, a seroprevalence of 11.5%  
153 (15/131; 95% CI = 6.0%-16.9%). All organ samples (lung samples (n=79), brain samples (n=  
154 35)) tested PCR negative for CDV (0/79; 95% CI = 0.00%-0.05%). According to our model  
155 selection, neither season, age, sex, or location was retained by most parsimonious models  
156 explaining exposure to either *T. gondii* or *Leptospira*.

157

158 This is the first known report of exposure of polecats to *T. gondii* in Britain though previous  
159 studies have detected *T. gondii* in polecats by PCR (Burrells *et al.*, 2013). The recorded  
160 seroprevalence of 71.8% (n=94/131) against *T. gondii* in polecats in this study is comparable  
161 to other studies of wild mustelids in Chile, 59% (n=43/73) in American mink and 77%  
162 (n=10/13) in the Southern sea otter (*Enhydra lutris nereis*) (Barros *et al.*, 2018), and wild  
163 carnivores in Spain, 67.4% (n=190/282) (Sobrino *et al.*, 2007). Although other studies have

164 found increasing exposure to *T. gondii* with age (Barros *et al.*, 2018; Sepúlveda *et al.*, 2011),  
165 this was not the case in the current study. This may be a result of age only being known for a  
166 relatively small subset of the study population (n=62). The most likely route of exposure of  
167 polecats to *T. gondii* is through consumption of infected prey. The majority of the polecat diet  
168 is made up of rabbits (Sainsbury *et al.*, 2020) which can be frequently infected with *T. gondii*  
169 cysts (Hughes *et al.*, 2008). While most *T. gondii* infections in mammals are usually sub-  
170 clinical, the parasite can be a significant pathogen in pregnant animals and young animals  
171 with an immature immune response, in which it may cause abortion or clinical toxoplasmosis  
172 (Burns *et al.*, 2003; Hollings *et al.*, 2013; Webster, 2001). While population effects are  
173 difficult to predict, high levels of exposure to this pathogen could potentially have an  
174 important impact on reducing fecundity and longevity in wild polecats (Webster, 2001), thus  
175 compromising population survival and recovery where reservoir species are locally abundant  
176 (Moinet *et al.*, 2010).

177

178 This is the first known report of exposure of polecats to *Leptospira* serovars in Britain with  
179 exposure to *Leptospira* serovars Bratislava, Saxkoebing and Icterohaemorrhagiae detected  
180 and an overall seroprevalence of 14.5% (n=19/131) recorded in this study. The three  
181 *Leptospira* serovars recorded in this study have reservoir hosts mainly consisting of rats and  
182 small rodents (further details in Supplemental Material 3) and exposure is likely through  
183 consumption of infected rodents or sharing the same environment. Most polecats  
184 seropositive for *Leptospira* serovars were also seropositive for *T. gondii*, 11.5% (n=15/131),  
185 likely a result of exposure to both pathogens from rodent reservoir hosts. The seroprevalence  
186 in the current study is lower than the 65.4% (n=87/133) reported in polecats in France  
187 (Moinet *et al.*, 2010) which may reflect differences in pathogen prevalence in reservoir hosts,  
188 or reduced contact with significant reservoir hosts in Britain through behaviour and dietary



189 preferences. The results from this study should also be regarded as the lower limit of the  
190 estimated seroprevalence, due to sample quality affecting detection of the lowest level of  
191 antibodies detectable by the assay (Supplemental material 2). Dietary analyses on polecats in  
192 Britain have shown that rabbits are the main prey source, and rodents make up a minor  
193 component of the diet (Sainsbury *et al.*, 2020). While there is no known data on the  
194 prevalence of *Leptospira* spp. in Britain's wild rabbit population, a quarter of wood mice  
195 (*Apodemus sylvaticus*) have been found to be infected with *Leptospira* spp. (Twigg, 2008)  
196 and 14-70% of brown rats (*Rattus norvegicus*) on UK farms were infected (Webster, Ellis  
197 and Macdonald, 1995). Wood mice are a common species in areas of preferred habitat of  
198 polecats such as the edges of woodland, hedgerows and field boundaries, and are preyed on  
199 by polecats (Birks, 1998; Birks, 2015). Polecats are also known to prey on brown rats whilst  
200 resting in farm buildings, particularly during winter (Birks, 1998). Infection of polecats with  
201 *Leptospira* serovars could potentially affect polecat population recovery through mortality  
202 and reduced longevity or fecundity. Current species specific information on whether polecats  
203 are incidental hosts of *Leptospira* spp., leading to potential acute renal or hepatic symptoms,  
204 or maintenance hosts, with potential reproductive and sub-clinical diseases, is limited (Ellis,  
205 2015; Schuller *et al.*, 2015).

206

207 Canine distemper virus may cause high mortality rates in the domestic ferret (Kiupel and  
208 Perpiñán, 2014) so the lack of detection of the virus in this study is reassuring for population  
209 recovery in Britain. Previous British studies have not detected CDV infection in other  
210 mustelids (Delahay and Frölich, 2000; Harrington *et al.*, 2012) though surveillance is limited.  
211 Reported cases in domestic dogs (*Canis familiaris*) in Britain are uncommon (SAVSNET,  
212 2018), suggesting that a wildlife reservoir is absent or CDV is circulating at low levels.  
213 However, vaccination against CDV is not a current requirement for imported dogs which

214 poses a potential risk for introduction to susceptible wild and domestic hosts in Britain. If  
215 CDV were to spill-over from domestic dogs into the polecat population and other wildlife  
216 species, it could result in mortality, immunosuppression and increased susceptibility to other  
217 diseases.

218

219 Evidence of exposure to *T. gondii* and *Leptospira* serovars in European polecats could  
220 potentially affect the recovery of polecat populations in Britain, through effects on mortality,  
221 longevity or fecundity. These effects are difficult to assess in the absence of long-term studies  
222 on pathogen prevalence and disease within the polecat population in Britain. More broadly,  
223 national studies of polecat range change have shown that polecats have been successfully  
224 recolonising their former range in Britain and so any limiting effects of exposure to *T. gondii*  
225 and *Leptospira* serovars have not been severe enough to prevent range expansion from taking  
226 place to date. Ongoing monitoring of pathogen exposure, disease and range expansion are  
227 required to quantify this risk for future polecat recovery.

228

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247

248 The data is available from the corresponding author.

249

250 On behalf of all authors, the corresponding author states that there is no conflict of interest.

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393 Table 1: The number of polecat carcasses found and submitted to this study between 2011-  
394 2016 from across Britain within each season and by sex.

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	<b>Autumn</b>	<b>Spring</b>	<b>Summer</b>	<b>Winter</b>	<b>TOTAL</b>
<b>Female</b>	8	7	13	4	32
<b>Male</b>	21	42	18	35	116 <sup>4</sup>
<b>Total</b>	29	49	31	39	148

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397 <sup>4</sup> The date of recovery of one male polecat was not available and is not recorded in the table.

398

399 Table 2: *Toxoplasma gondii* titre results for 131 polecats tested in this study and the  
400 interpretation of the titres

401

<b><i>T. gondii</i> Antibody Titre</b>	<b>Number of Polecats</b>	<b>Interpretation (presence or absence of <i>T. gondii</i> antibodies)</b>
<1:16	16	Absence
1:16	12	Absence
1:32	9	Presence (borderline <sup>5</sup> )
1:64	28	Presence
1:128	17	Presence
1:256	16	Presence
1:512	16	Presence
≥1:1024	17	Presence

402 <sup>5</sup> Borderline results were not included in the exposed category.

403

404

405 Table 3: The number of polecats exposed to each *Leptospira* serovar and the number of  
 406 polecats exposed overall. The seroprevalence and 95% confidence intervals are also included.  
 407

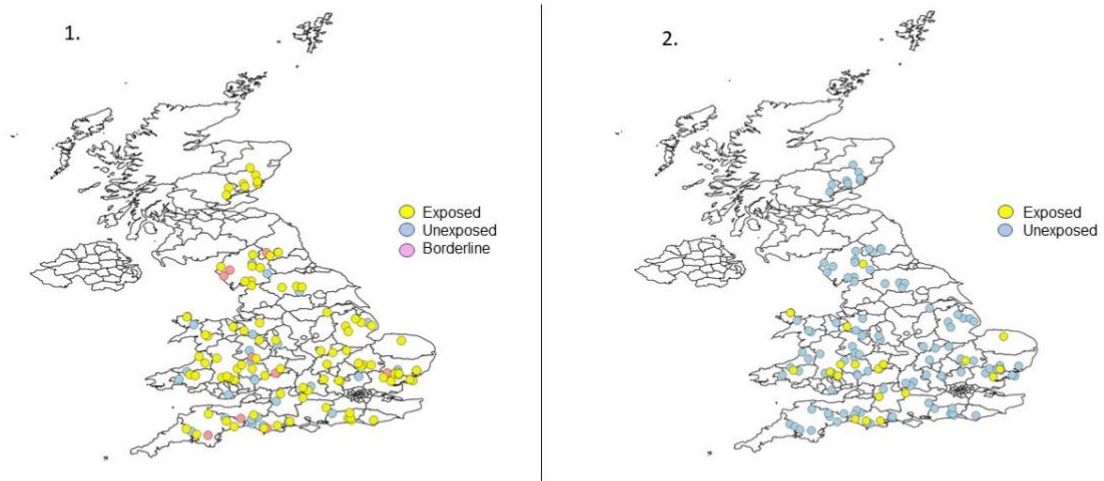
	<b>Exposed</b>		<b>Total</b>	<b>Unexposed</b>	<b>Seroprevalence</b>	<b>95% CI</b>
	<b>MAT titre</b>					
	<b>1/10</b>	<b>1/30</b>				
Bratislava	0	10	<b>10</b>	121	7.6% (10/131)	3.2- 12.0
Saxkoebing	1	7	<b>8</b>	119	6.3% (8/127) <sup>6</sup>	1.9- 10.7
Icterohaemorrhagiae	0	2	<b>2</b>	129	1.5% (2/131)	0- 3.5
<i>Leptospira</i> spp.			<b>19</b>	112	14.5% (19/131) <sup>7</sup>	8.6- 20.4

408 <sup>6</sup> Four samples were not tested for Saxkoebing, Altodouro, Grippotyphosa or Javanica, and one sample was not tested for Altodouro or  
 409 Grippotyphosa. This was either because there was insufficient serum left to test or the quality of the remaining sample was too poor.

410 <sup>7</sup> One polecat showed evidence of exposure to *Leptospira* serovars Saxkoebing and Bratislava.

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413

414 **Fig. 1** Maps to show the presence and absence of a serological response against *Toxoplasma gondii*

415 (1.) and *Leptospira* serovars (2.) in polecats (*Mustela putorius*) collected across Britain (2011-2016).

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Maps drawn in qGIS

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427 Supplemental Material 1:

428 Details of how European polecats (*Mustela putorius*) in this study were categorised as adults  
429 or juveniles.

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431 For the subset of polecats whose age was estimated, they were categorised as an adult if their  
432 age was estimated to be over 12 months old. If their age was estimated at less than 12 months  
433 old, the estimated birth month was calculated by subtracting their age from their finding date.  
434 If the carcass was found in the year after which the polecat was estimated to have been born,  
435 the carcass was classified as an adult. This classification is appropriate because polecats can  
436 breed from the year following that of their birth (Blandford, 1987). Polecats were classified  
437 as juveniles if they were less than 12 months old and the carcass was found in the same year  
438 that the animal was born.

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449 Supplemental Material 2:

450 Methods used to test serum from European polecats (*Mustela putorius*) in this study for  
451 antibodies to *Toxoplasma gondii* and *Leptospira* serovars, and the PCR method used to test  
452 tissue samples for the presence of Canine Distemper Virus RNA.

453

454 The Latex Agglutination Test (LAT) (MAST Toxoreagent Toxoplasma Test, RST7001, Mast  
455 Group Ltd, Bootle, UK), was used following the manufacturer's instructions, to screen the  
456 polecat sera for antibodies against *Toxoplasma gondii*. Three samples were initially sent to  
457 the laboratory to assess whether the quality of the samples would affect the ability of the LAT  
458 to detect *T. gondii*. The results were not affected by the sample quality. Controls for the test  
459 included the manufacturer's own positive control of human serum and a known feline  
460 positive. Based on the manufacturer's recommendations, a titre of 1:64 or greater indicated  
461 the presence of *T. gondii* antibodies and was indicative of exposure. The LAT is not host  
462 species specific so it can be used in a wide variety of applications. The test has a sensitivity  
463 of 93.8% and a specificity of 94.1%.

464 The Leptospirosis laboratory at the Agri-Food and Biosciences Institute Stormont  
465 Belfast carried out an in-house *Leptospira* Microscopic Agglutination Test (LMAT) to screen  
466 sera for antibodies against a panel of twelve *Leptospira* serovars: *Leptospira interrogans*  
467 *Autumnalis*, *Bratislava*, *Canicola*, *Icterohaemorrhagiae*, *Pomona* and *Saxkoebing*; *Leptospira*  
468 *borgpetersenii* *Ballum*, *Hardjo bovis*, *Tarasovi*, *Javanica* and *Altodouro*; *Leptospira kirshneri*  
469 *Grippotyphosa*. The LMAT is used all over the world, on a very wide variety of species, and  
470 is widely accepted as a principle tool in *Leptospira* spp. seroprevalence studies and routine  
471 surveillance. While the LMAT is largely specific to the serovar being tested because there  
472 can be a high degree of cross-reactivity between serovars, the panel included the main

473 serogroups. The laboratory had not tested sera from polecats before and so there is no  
474 sensitivity data available.

475       Blood samples were spun down further or filtered as necessary. However, it is still  
476 possible that samples with a low level of antibodies (1/10) may not have been detected due to  
477 sample quality. The test was performed by preparing two dilutions (1/30 and 1/300) of each  
478 serum sample, followed by the addition of equal volumes of each of the twelve  
479 antigens. After incubation at 28°C for two hours, the test was read in-directly and a titre was  
480 given where there was sufficient antibody present to lyse or agglutinate 50% of the organisms  
481 present (75% for 1/10 due to auto-agglutination). Polyclonal short-term rabbit sera were used  
482 as positive controls. Titres of 1/10 or greater were considered significant and were indicative  
483 of exposure. For each serum sample, the serovar with the highest titre was considered to be  
484 the infecting serovar, and serovars from the same serogroup with lower titres were considered  
485 to be cross reactions and were not included in the results. The subjectivity of the LMAT is  
486 consistent within a laboratory.

487

488 Serum was used to detect exposure to CDV using a serum neutralisation assay, developed by  
489 the Veterinary Diagnostic Services at the University of Glasgow, and a viral pseudotype  
490 assay (Logan *et al.*, 2016), but the tested samples were inconclusive, likely due to haemolysis  
491 of the serum samples. Therefore, PCR was used on selected organ samples to assess the  
492 presence or absence of CDV nucleic acid. DNA was extracted for CDV PCR and DNA  
493 integrity checked by testing for detection for a host gene. A novel real-time RT-PCR assay,  
494 targeting the nucleocapsid gene, was utilised (a manuscript is in preparation for the primers).  
495 Degeneracy was incorporated into primers to ensure all potential targets were amplified.  
496 Total nucleic acid was extracted from all submitted samples using the TRIzol reagent  
497 (ThermoFisher Scientific, Waltham US). Commercially available RT-PCR master mixes

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498 were utilised (Qiagen, Manchester, UK) and template RNA (1ng/μl) and primers (10pmol/μl)  
499 added. A dilution series of CDV-positive control RNA, and a negative RNA sample were run  
500 alongside the samples. The following cycling conditions were followed: 50°C for 5min, 95°C  
501 15mins, then 45 cycles of (94°C 30 sec; 45°C 10sec; 50°C 15sec; 72°C 1min) followed by a  
502 final extension of 72°C for 7mins. Reactions were analysed using Stratagene's MXPro  
503 software (Santa Clara, US).

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519 Supplemental Material 3:

520 Reservoir hosts for *Leptospira* serovars Bratislava, Saxkoebing and Icterohaemorrhagiae.

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522 Reservoir hosts for *Leptospira* serovar Bratislava are the pig (*Sus scrofa domesticus*), horse

523 (*Equus ferus caballus*), hedgehog (*Erinaceus europaeus*) (Webster, Ellis and Macdonald,

524 1995) and small rodents (the yellow necked mouse (*Apodemus flavicollis*) and wood mouse

525 (*A. sylvaticus*)) (Milas *et al.*, 2013); for *Leptospira* serovar Saxkoebing reservoir hosts are

526 small rodents (yellow necked mouse and wood mouse) (Little, Stevens and Hathaway, 1986)

527 and for *Leptospira* serovar Icterohaemorrhagiae reservoir hosts are brown rats *Rattus*

528 *norvegicus* (Ellis, 2015).

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