Short Communication

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

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Abstract

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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The data is available from the corresponding author.

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

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

	Autumn	Spring	Summer	Winter	TOTAL
Female	8	7	13	4	32
Male	21	42	18	35	116[4]
Total	29	49	31	39	148

[4] The date of recovery of one male polecat was not available and is not recorded in the table.

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

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

[5] Borderline results were not included in the exposed category.

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

408 6 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 7 One polecat showed evidence of exposure to *Leptospira* serovars Saxkoebing and Bratislava.
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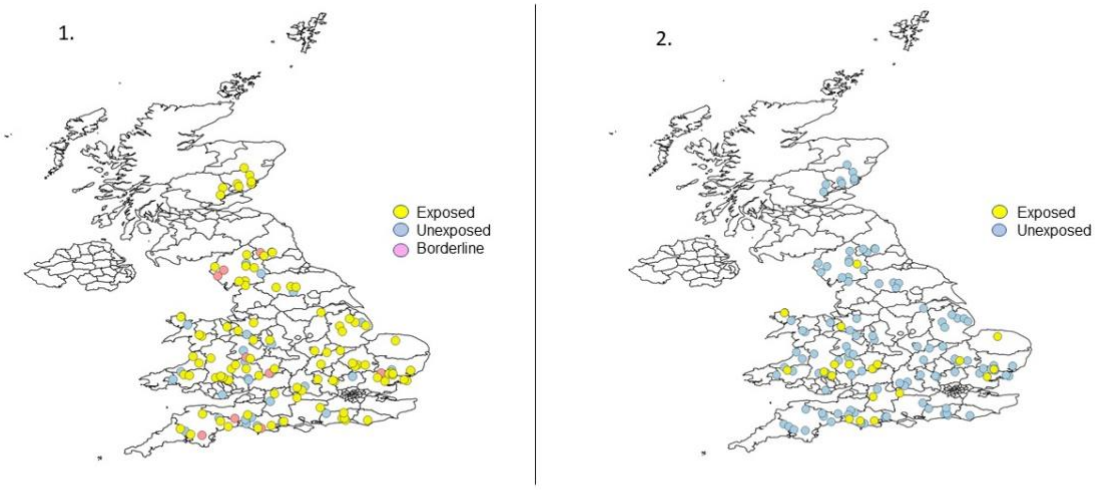

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414 **Fig. 1** Maps to show the presence and absence of a serological response against *Toxoplasma gondii*
415 (1.) and *Leptospira* serovars (2.) in polecats (*Mustela putorius*) collected across Britain (2011-2016).
416 Maps drawn in qGIS
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Supplemental Material 1:

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

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

Supplemental Material 2:

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

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

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

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

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

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

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

Supplemental Material 3:

Reservoir hosts for *Leptospira* serovars Bratislava, Saxkoebing and Icterohaemorraghiae.

Reservoir hosts for *Leptospira* serovar Bratislava are the pig (*Sus scrofa domesticus*), horse (*Equus ferus caballus*), hedgehog (*Erinaceus europaeus*) (Webster, Ellis and Macdonald, 1995) and small rodents (the yellow necked mouse (*Apodemus flavicollis*) and wood mouse (*A. sylvaticus*)) (Milas *et al.*, 2013); for *Leptospira* serovar Saxkoebing reservoir hosts are small rodents (yellow necked mouse and wood mouse) (Little, Stevens and Hathaway, 1986) and for *Leptospira* serovar Icterohaemorraghiae reservoir hosts are brown rats *Rattus norvegicus* (Ellis, 2015).

Supplemental Material References

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