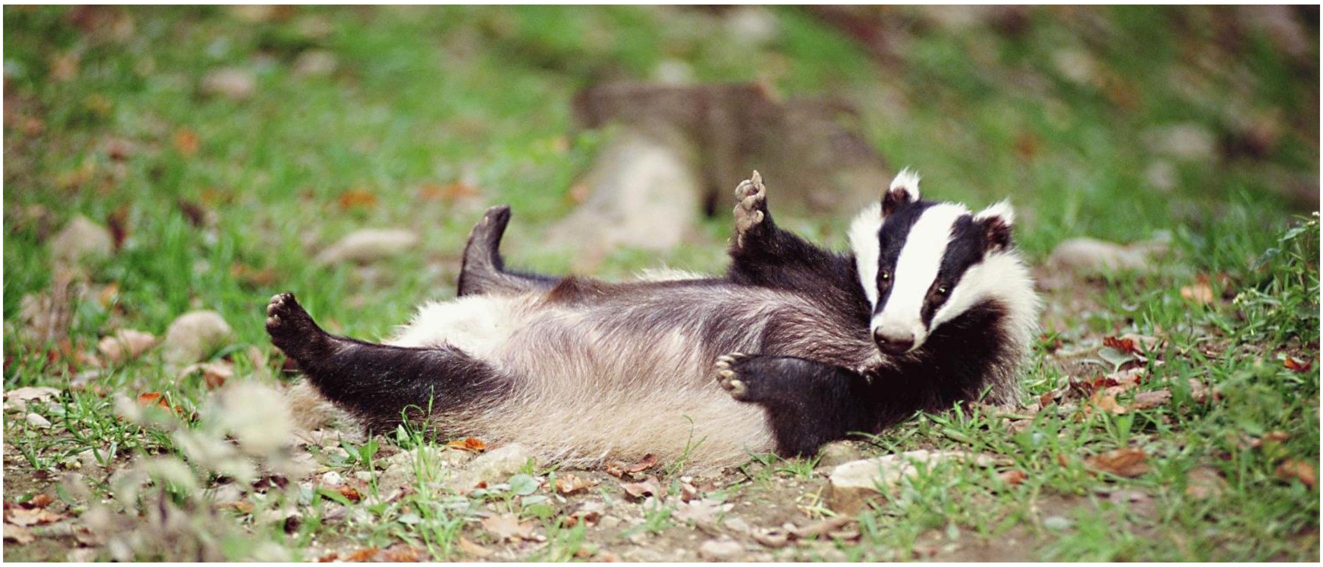

Internship report for the People's Trust for Endangered Species

# Evaluating the prevalence and clinical significance of sexually transmitted diseases (STDs) in a European badger *Meles meles* population: Potential implications for female reproductive biology and immunology.

By

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## Abstract

My internship project aimed to investigate the presence and prevalence of two potential STDs of European badgers, *Meles meles*: *Chlamydia* and *Mustelid herpesvirus*-1(MusHV-1). Due to their promiscuous nature, badgers represent a good model species to study the often neglected epidemiology of sexually transmitted diseases (STDs); research which is then also applicable to the conservation of other mustelid species, many of which are threatened or endangered. The study was carried out in Wytham Woods, Oxfordshire, where the resident badger population has been studied intensively by the Wildlife Conservation Research Unit (WildCRU) for the past 30 years. During a routine trapping session, 100 genital swab samples were collected from 98 individuals (27 males, 71 females). All samples (100/100) tested negative for *Chlamydia* species. Prevalence of MusHV-1 in genital swabs, however, was 39% (15/38). This is in contrast to the results of a previous study, where the blood of 98.1% of badgers (354/361) in this population was infected with MusHV-1. The detection of MusHV-1 in the female reproductive tract strongly indicates a sexual, and potentially also a vertical (i.e. mother to offspring), route of transmission.

## Introduction

Infectious diseases play an important role in shaping wildlife populations, and can cause extreme localized declines (Kat *et al.* 1996; see review by Macdonald, 1996; Roelke-Parker *et al.* 1996; Randall *et al.* 2004; McCallum *et al.* 2009), in some cases threatening the very survival of endangered species (Leroy *et al.* 2004; Randall *et al.* 2006). Understanding their prevalence, transmission dynamics and health implications is therefore key to ensuring effective conservation management (Murray *et al.* 1999; Funk *et al.* 2001; Woodroffe *et al.* 2004; McCallum *et al.* 2009).

Particularly sexually transmitted diseases (STDs) are often overlooked, and their importance, especially in wildlife population dynamics, underrated (Lockhart *et al.* 1996; Knell and Webberley, 2004). The adverse effects of STDs have long been recognized, and include diseases of the reproductive tract (Kellen and Lindegren, 1971; McColl *et al.* 1984; Girjes *et al.* 1993; Blunden *et al.* 1998), host sterility, and reduced host fecundity (Hurst *et al.* 1995; Martin and Handasyde, 1999). Despite such concerning implications for population health and viability, empirical research on the prevalence of STDs, and their impacts on wildlife populations is negligible.

### The European badger

The European badger, *Meles meles,* is a medium sized, fossorial carnivore of the *Mustelidae*, native to most of Europe and parts of the Middle East. At low densities, badgers are primarily solitary, but can form large social groups of up to 30 individuals at higher population densities (Johnson *et al.* 2000; Johnson *et al.* 2002). Staple foods of badgers include earthworms, fruit, insects, mammals and cereals, varying according to their geographical range (reviewed by

Roper 1994; Roper 2010). They face ongoing persecution from badger baiting, and farmers due to their role in crop destruction and transmission of bovine tuberculosis (bTB) to cattle in the UK (Hutching and Harris, 1999), making them one of the most protected species in Britain (Badger Act 1973; Schedule 5 of the Wildlife and Countryside Act 1981; Protection of Badgers Act 1992).

In general, disease studies of the European badger are limited, and predominantly investigate non-sexually transmitted pathogens from a diverse taxonomy (see review by Hancox 1980; *Rabies:* Serokowa, 1968; *Canine Distemper Virus:* Van Moll *et al.* 1995; *Eimeria melis* and *Isospora melis:* Newman *et al.* 2001; helminths: Torres *et al.* 2001; *Salmonella:* Wilson *et al.* 2003; *Mycobacterium bovis:* Carter *et al.* 2007; *Trypanosoma pestanai:* Lizundia *et al.* 2011; *Polyomavirus:* Hill *et al.* 2015). European badgers, however, represent an interesting wildlife model to study STD-epidemiology, as they are group-living and have a promiscuous, polygynandrous mating system (Dugdale *et al.* 2007; Dugdale *et al.* 2008; Annavi *et al.* 2014a). Unexpectedly, despite their promiscuity, fecundity in badgers is low. In the study population, only 45% of adult females bred successfully between 1987 and 2010, and average litter size was 1.4 cubs per annum (Dugdale *et al.* 2007; Annavi *et al.* 2014a; Macdonald *et al.* 2015). This low reproductive success has been partly explained by body condition whereby females with sufficient fat reserves can invest more in embryo development (Woodroffe, 1995). By investigating changes in litter sizes during the reproductive cycle, it is estimated that pre-natal mortality in badgers might be as high as 15-20% (Wandeler and Graf, 1982; Anderson and Trewhella, 1985). Research strongly implies that failure to develop and implant blastocysts (Anderson and Trewhella, 1985), loss of embryos (Cresswell *et al.* 1992; Page *et al.* 1994) through reabsorption (Wandeler and Graf, 1982; Anderson and Trewhella, 1985; Woodroffe and Macdonald, 1995; Yamaguchi *et al.* 2006) and abortion of litters (Anderson and Trewhella, 1985; Page *et al.* 1994) are common reproductive failures in wild badgers. Studies on badger population dynamics (Macdonald *et al.* 2002; Macdonald *et al.* 2009) and genetics (Annavi *et al.* 2014a; Sin *et al.* 2014a), however, typically discuss reproductive failure in view of environmental (Macdonald and Newman, 2002; Macdonald *et al.* 2010; Nouvellet *et al.* 2013; Annavi *et al.* 2014b) and behavioural factors (Woodroffe and Macdonald, 1995; Woodroffe and Macdondald, 2000; Dugdale *et al.* 2008), disregarding potential involvement of STDs.

## Common STDs: *Chlamydia* and herpesviruses

Of particularly high concern in animal welfare and productivity are the *Chlamydia* species *C. pecorum* and *C. arbortus*, which cause reproductive diseases in livestock on a global scale (Aitken and Longbottom, 2007; Berri *et al.* 2009). Infection from *C. arbortus* incites premature births and abortions in sheep and goats (Longbottom and Coulter, 2003), having accounted for a 45% loss of lambs in the UK (Aitken *et al.*1990). Although *Chlamydia* spp. are most prevalent in ruminants (Lockhart *et al.* 1996), they inflict severe diseases on a wide range of hosts, including humans (Grayston *et al.* 1993; Wardrop *et al.* 1999; Bodetti and Timms, 2000; Bodetti *et al.* 2002), with some *Chlamydia* spp. showing an expanding host range (Wardrop *et al.* 1999; Bodetti *et al.* 2002; Schrenzel *et al.* 2008). Given that *Chlamydia* spp. frequently cause STDs in other mammals, including humans (Christophersen, 1988), we propose that *Chlamydia* infection in the reproductive tract of badgers may play an important role in explaining the high rates of reproductive failure observed.

Herpesviruses have also been linked to abortion, stillbirth, and foetal resorption in domestic animals (Ayers *et al.* 1989; see reviews by Crabb and Studdert, 1995 and Smith, 1997; e.g. Muylkens *et al.* 2007; LeCuyer *et al.* 2015) with additional evidence of abortion (Montali *et al.* 1985; Wolff *et al.* 1986; Guo *et al.* 2014) and disease of the genital tract (Blunden *et al.* 1998) in wildlife species, as well as humans (Leach *et al.* 1994; Whitby *et al.* 1999). A novel herpesvirus (BadHV), later classified as *Mustelid herpesvirus*-1 (MusHV-1), has been isolated from the lungs of a European badger in southwest England (Banks *et al.* 2002) and Hungary (Dandár *et al.* 2010). Using a specific polymerase-chain reaction (PCR) assay, reported prevalence of MusHV-1 in the blood was high throughout the UK (southwest England: 95% [18/19]; Ireland: 100% [10/10]; and in bodily tissues, see King *et al.* 2004). In the high density population at Wytham Woods, Oxfordshire used in this study, high prevalence of MusHV-1 was confirmed from blood samples (98.1% [354/361] over 2 years; Sin *et al.* 2014a), but knowledge on the pathogenesis of MusHV-1 in badgers is limited. Symptomology has only been reported in one study regarding lesions in a badger's lungs (Banks *et al.* 2002). Nevertheless, MusHV-1 is closely related to the gammaherpesviruses (e.g. *Equid herpesvirus*-2*:* EHV-2, Allen and Murray 2004) that are known to cause immunosuppression, respiratory disease (Fu *et al.* 1986), potentially chronic pulmonary disease (Schlocker *et al.* 1995), abortion (see review by Smith, 1997; Montali *et al.* 1985; Wolff *et al.* 1986; review by Crabb and Studdert, 1995; Guo *et al.* 2014; LeCuyer *et al.* 2015), diseases of the female reproductive tract, and scrotal vascular damage (Blunden *et al.* 1998). Considering MusHV-1 has been isolated from multiple bodily media, we posit that MusHV-1 also infects the genital tract of badgers (e.g. human herpesviruses: including another gammaherpesvirus: HHV-8 (Leach *et al.* 1994; Whitby *et al.* 1999). We thus predict that MusHV-1 can have adverse effects on the somatic and reproductive fitness of badgers.

For the plethora of reasons discussed, STDs and their transmission are likely to be significant in the European badger. This study will therefore investigate for the first time the presence and prevalence of MusHV-1 and *Chlamydia* in the genital tract of wild European badgers to increase our understanding of the clinical significance of these pathogens as wildlife STDs.

## Materials and Methods

*Study population and sample collection*

The study was conducted in a high-density badger population [44.55 ± 5.37 (SE) badgers/km$^2$; Annavi *et al.* 2014a, Macdonald *et al.* 2015] in Wytham Woods, a 424-ha mixed woodland in Oxfordshire, UK (51°46026N, 1°19019W; for description of study site see Savill *et al.* 2010; for details of the badger population see Macdonald *et al.* 2015) which has been researched extensively since the 1970s (Macdonald *et al.* 2015).

As part of an ongoing long-term population study, which started in 1987 (for details see Macdonald and Newman, 2002; Macdonald *et al.* 2015), saturation trapping is carried out routinely over two weeks in May/June (spring), and in August (summer) and November (autumn). At first capture (usually as cubs), all badgers receive a permanent unique tattoo in the left inguinal region, which enabled individual identification and age classification as cub (< 1 year), yearling (< 2 years, sexually immature) or adult (≥ 2 years; see Tinnesand *et al.* 2015). Tattoo, sex and social group membership (determined by bait-marking: Delahay *et al.* 2000) were recorded, and body condition was categorised as 1 = emaciated to 5 = very good condition (Speedy, 1980; Buesching *et al.* 2009). Reproductive status of adult females was deduced from vulva condition and classified as oestrus (vulva swollen and moist), or non-oestrus (vulva flat and dry). For each female, the length and diameter of each teat was measured to infer reproductive success (Dugdale *et al.* 2011). Vaginal and penal swabs were obtained during the spring and summer trappings of 2015, using pre-sterilised woodstick shaft cotton tip swabs and all samples were frozen immediately.

## Laboratory Analysis

*Pathogen Screening*

*Mustelid herpesvirus-1 and Chlamydia.* DNA was extracted from swabs as recommended by the QIAamp DNA Mini Kit. A generic panherpesvirus polymerase chain reaction (PCR) was used to test for gammaherpesviruses and alphaherpesviruses. The presence of *Chlamydia* species was investigated using quantitative real-time PCR (Ehricht *et al.* 2006; Merdja *et al.* 2015). A sample was considered negative if the fluorescent signal did not increase after 45 cycles for a minimum of two of the three replicates.

## Results

*Chlamydia*

All 100 genital swab samples (from 98 individual badgers) collected between the period of May/June (i.e. ‘spring’) and August (i.e. ‘summer’) of 2015 were negative for *Chlamydial* DNA using real-time PCR.

*Herpesviruses*

Only female spring samples (35 adults, 3 cubs) were tested for prevalence of herpesviruses. A total of 15 samples (14 adults, 1 cub; i.e. 39%) were confirmed positive for gammaherpesvirus DNA, using a generic panherpesvirus PCR, which was then characterized as MusHV-1 using DNA sequencing. There was no detection of alphaherpesviruses in the DNA of swab samples.

There was no significant difference in mean body condition of infected badgers compared to uninfected badgers (Independent two-sample t-test: $t_{36} = 0.8$, $p = 0.42$), and infection status did not affect female reproductive condition (Fisher's Exact test, p= 1), with the same numbers of infected (10/14= 71%) and uninfected individuals (15/21= 71%) being in oestrus, compared to non-oestrus (infected badgers: 4/14= 29%; uninfected badgers: 6/21= 29%).

Statistically, there was no significant effect of herpes-infection on female reproductive success as determined through signs of recent lactation (Chi-squared test, $X^2 = 0.004$, df = 1, n = 35, p >0.05). Nevertheless, there was a slight trend showing that the number of infected badgers that had lactated was marginally lower (5/13=38%) than the number of uninfected badgers (9/21=43%), and the number of infected badgers that had not lactated was marginally higher (8/13=62%) than the number of uninfected badgers (12/21=57%).

Twenty percent of setts (5/25 setts, belonging to 4 social groups), from where multiple females were tested for herpesviruses, reported infected as well as uninfected badgers.

## **Discussion**

This is the first systematic study investigating the occurrence and prevalence of *Chlamydia* and a herpesvirus in the genital tract of European badgers. While we found no evidence of *Chlamydia* infection, MusHV-1 was reported in the vagina of 40% (14/35) of adult females and 33% (1/3) of female cubs, supporting our hypothesis. The presence of MusHV-1 in the genital tract of adult females thus suggests a sexual transmission route. Indeed, the badger's promiscuous mating system whereby both males and females mate with multiple partners, would facilitate sexual transmission and the spread of MusHV-1. Nevertheless, counter to our expectations, genital infection with MusHV-1 neither manifested itself in lowered body condition nor adverse affects on reproductive success and condition.

Previously, MusHV-1 has been identified in the lungs of a young adult female badger (Banks *et al.* 2002) and in the blood of 98.1% of individuals (adults and cubs of both sexes) from the study badger population (Sin *et al.* 2014a). As the generic panherpesvirus PCR could have reduced sensitivity in detecting this particular herpesvirus in genital swab samples compared to conventional PCR (Bernhard Ehlers, *pers. comm.*), it is plausible that limitations of the laboratory methods could have produced false negative results, explaining the lower prevalence of MusHV-1 in the reproductive tract (15/38= 39%) compared to blood (354/361= 98.1%). In the previous study, the blood samples of 361 badgers were collected over different seasons from spring (June) 2009 to January 2010 compared to the 38 tested genital swabs collected over 2 weeks in spring 2015. Seasonal effects on the transmission dynamics of MusHV-1 could explain the different infection rates of MusHV-1 in the blood and genital tract. Intensity of MusHV-1 infection in the blood was shown to be higher in adults during the spring, compared to the winter (Sin *et al.* 2014a), possibly due to increased bite-wounding during the post-partum mating period in February/March. Therefore we might expect the prevalence of MusHV-1 in the genital tract of female badgers to be higher during the peak mating season in winter compared to spring. Sexual transmission of MusHV-1 is likely to be more variable due to differential mating strategies of individuals. Cubs, however, had higher intensity of infection in the blood than adults (Sin *et al.* 2014a), most likely because they are still acquiring immunological B and T cells, thus making them more susceptibile to infection (Sol *et al*. 2003). Even though bite-wounds from attempted infanticide (Macdonald *et al.* 2015) could contribute to infection, bite wounds in cubs are less common (Delahay *et al.* 2006). It is more likely that MusHV-1 infects the blood of cubs vertically via the placenta or their mother's milk, as implied in cows (Donofrio *et al.* 2000). The lower prevalence of MusHV-1 in the genital tract of cubs compared to adults, can be explained by cubs not engaging in sexual contact, reaffirming evidence that MusHV-1 is (also) transmitted sexually.

All 38 females (infected and uninfected individuals) in this study were of similar body condition, reproductive condition and had similar breeding success (35 badgers excluding 3 cubs). Furthermore, the majority of infected badgers also shared social groups and setts, indicating that other factors are likely to play a part in predisposing some females to MusHV-1 infection more than others. Potentially, infected adult females are more promiscuous and increasingly exposed to MusHV-1 through mating, where promiscuity can be related to high prevalence of an STD (Webberley *et al.* 2002). Alternatively, variability in female mate choice could explain the variability of female infection rates. Both 'risky' females, those that mate indiscriminately, and 'risk-averse'females could persist in a population in the presence of an STD when risky behaviour incurs some genetic benefit (Boots and Knell, 2002). Namely, this benefit would be increased reproductive success from multiple matings at the risk of exposure to STDs. Female badgers have been shown to select mates with a similar Major Histocompatbility Complex (MHC), a selection of genes responsible for immuno-functioning (Sin *et al.* subm), potentially gaining immunity advantages for their offspring in favour of genetic diversity (Nowak *et al.* 1992). Consequently, some adult females could have succumbed to infection or reinfection of MusHV-1 in the genital tract due to low immuno-functioning attributable to their genetic make-up, whereby specific alleles can increase susceptiblity to pathogens (Sin *et al.* 2014a).On the other hand, 'choosy', risk-averse females may potentially decrease their mating opportunities, but could gain direct fitness benefits by mating selectively with genetically 'fit' males, whilst simultaneously minimizing their own as well as their offspring's exposure to STDs. Males are known to move frequently between social groups/setts to forage and mate (Macdonald *et al.* 2008). In-fact, ca. 50% of litters in Wytham Woods are sired by extra-group males (Dugdale *et al.* 2007; Annavi *et al.* 2014a), potentially explaining as to why infected females are fairly widespread among social groups and setts. Mate choice in badgers, however, is very cryptic, and thus males need to be investigated as a source of renewed infection to females.

Reactivation of gammaherpesviruses can occur through concomitant infections (Widén *et al.* 2012), stress, hormone changes, and immunosuppression (e.g. Winkler *et al.* 1999; experimental study in Tryland *et al*. 2009). In young cubs, extreme weather conditions commonly lead to malnutrition and high prevalence of parasitic coccidiosis (Newman *et al.* 2001; Nouvellet *et al.* 2013), the combination of which cause oxidative stress (Bilham et al. 2013). The resultant production of oxygen-free radicals can damage lipids, proteins and DNA. Protective enzymes and molecules, called 'antioxidants', help to combat this stress. Interestingly, badger cubs have been shown to have the same antioxidant capacity as young adults (Bilham *et al.* 2013). Therefore, low prevalence of MusHV-1 in the genital tract of cubs could potentially be explained in part by their comparatively high levels of antioxidants. Consequently, cubs with lower than average antioxidant capacity would likely display increased susceptibility to co-infections and potentially death during unfavourable environmental conditions. A related herpesvirus, the *Equid herpesvirus*-1 (EHV-1), has been shown to cause death of newborn Grevy's zebra (*Equus grevyi*: Wolff *et al.* 1986), supporting this hypothesis. Increased screening of female adults and cubs, coupled with genetic pedigree analysis, is needed to better understand the prevalence in both age-classes and transmission routes of MusHV-1. Additionally, life-history traits such as immunological, stress and co-infection profiles also need to be investigated in relation to MusHV-1 infection status to increase understanding of its pathogenesis in badgers. It is likely that interplaying genetic, behavioural and transmission factors are predisposing both, adults and cubs, to MusHV-1 infection. Clearly, considerable investigation into the complex epidemiology of this STD is required to understand mechanisms driving MusHV-1 infection in the genital tract at both the individual and the population level.


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