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**Population screening for herpesviruses in the genital tract of badgers (*Meles meles*) reveals wide-spread infection with MusGHV-1**

Alice Kent<sup>1</sup>, Bernhard Ehlers<sup>2</sup>, Tom Mendum<sup>3</sup>, Chris Newman<sup>1</sup>, David W. Macdonald<sup>1</sup>, Mark Chambers<sup>3</sup>, Christina D. Buesching<sup>1\*</sup>

<sup>1</sup>Wildlife Conservation Research Unit, Dept. of Zoology, University of Oxford, The Recanati-Kaplan Centre, Tubney House, Abingdon Road, Tubney, Abingdon OX13 5QL, UK

<sup>2</sup>Division 12 “Measles, Mumps, Rubella and Viruses affecting immune-compromised patients”, Robert Koch Institute, Berlin 13353, Germany

<sup>3</sup>School of Veterinary Medicine, Faculty of Health and Medical Sciences, University of Surrey, Guildford, GU2 7XH

\* Corresponding author: Dr. Christina D. Buesching

email: christina.buesching@zoo.ox.ac.uk

phone: +44 - (0)1865 - 611100

Fax: +44 – (0)1865 – 611101

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## Abstract

Sexually transmitted diseases (STDs) can be important drivers of population dynamics due to their negative impacts on reproduction. Yet, screening for STDs, especially in wildlife populations, remains widely neglected. Using the promiscuous, polygynandrous European badger, *Meles meles*, as a model, we investigated the presence and prevalence of herpesviruses (HV) in a wild high-density population, and analysed potential correlation between HV-infection status and impaired somatic fitness as well as potential links to female reproductive condition. We collected n=98 genital swabs (71 females: 51 adults and 20 cubs; 27 males: 26 adults and 1 cub) during spring and summer 2015. Using a polymerase chain reaction (PCR) specific for a mustelid alpha-HV, all genital swab samples tested negative. In a panherpes PCR, a gamma-HV was found in 55 % (54/98: 39 adults and 15 cubs), identified as Mustelid gammaherpesvirus 1 (MusGHV-1) using DNA sequencing. This contrasts with the results of a previous study, which reported MusGHV-1 in 98 % of blood samples (354/361) taken from 218 badgers in this same population using PCR. The detection of MusHV-1 in the female reproductive tract strongly indicates a sexual, and likely also a vertical, route of transmission. Our results potentially indicate that genital HV may correlate with impaired future reproductive success of females, but because reproductive failure can have many reasons in badgers, the causative link of this negative correlation remains to be investigated.

**Keywords:** *Alphaherpesvirinae*, *Betaherpesvirinae*, *Gammaherpesvirinae*, MusAHV-1, Mustelid herpesvirus, Sexually Transmitted Diseases, horizontal/ vertical transmission, reproductive success

## Introduction

Sexually transmitted infectious diseases (STDs) play an important role in shaping wildlife populations, and can cause extreme localized declines (Knell & Webberly 2004), in some cases threatening the very survival of endangered species (McCallum *et al.* 2009; Polkinghorne *et al.* 2013). Transmission of STDs can occur either ‘horizontally’, between an infected mate and an uninfected partner, and/ or ‘vertically’, between mother and offspring (Lockhart *et al.* 1996), providing important implications for their epidemiology. Understanding transmission dynamics, prevalence and health implications of STDs are therefore key to ensuring effective conservation management (Funk *et al.* 2001).

The *Herpesviridae* is one of the largest and most diverse virus families (Davison 2010), currently comprising 89 species (<https://talk.ictvonline.org/taxonomy/herpes>) that are morphologically distinct from other viruses, featuring a linear double-stranded DNA of 125-250 kbp surrounded by an icosahedral capsid. They are classified in three subfamilies, *Alphaherpesvirinae*, *Betaherpesvirinae* and *Gammapherpesvirinae*, and can infect a wide range of mammals, reptiles and birds (Widén *et al.* 2012), often causing diseases of the reproductive tract that result in decreased host fecundity or sterility (Smith 1997), or lowered somatic fitness (Winkler *et al.* 1999). Many of these venereal herpesvirus (HV) infections have been shown to be sexually transmittable either horizontally and/ or vertically (Corey & Handsfield 2000).

In a review, Lockhart *et al.* (1996) reported 200 STDs in 27 orders of mostly vertebrate hosts, including 14 HV infections in 4 wild and 10 domestic species. Nevertheless, despite the implications for population health and viability, empirical research on the prevalence and impacts of sexually transmitted HV in wildlife

75 populations is negligible. Amongst members of the terrestrial carnivorans, alpha-HV  
76 are common, and Canid HV-1 has been linked to genital ulcers, infertility and  
77 abortion in domestic dogs (Anvik 1991), with abortion being reported after artificial  
78 inoculation (Smith 1997), while the closely related Felid HV-1 causes respiratory and  
79 ocular disease in wild and domestic felids (Evermann *et al.* 1993; Kang & Park  
80 2008). Gamma-HV have been reported in six wild terrestrial carnivorans, four of  
81 which are mustelids infected by species-specific pathogens, with only three species  
82 displaying clinical signs (Cabello *et al.* 2013). Interestingly, beta-HV have thus far not  
83 been reported in any carnivoran species (Davison 2010).

84 European badgers, *Meles meles*, present an interesting wildlife model to study HV  
85 epidemiology, as they are group-living and have a promiscuous, polygynandrous  
86 mating system (Dugdale *et al.* 2007). Their main mating season is restricted to a  
87 post-partum estrus (late January – mid-March) with possibility of superfecundation  
88 and superfetation during delayed implantation (Yamaguchi *et al.* 2006). Altricial cubs  
89 are born around mid-February and stay in the underground sett until weaning at 6-8  
90 weeks of age (Macdonald *et al.* 2009). Despite their promiscuity, however, fecundity  
91 in badgers is low (Macdonald *et al.* 2009): On average, ca. 45% of adult females  
92 breed successfully in high-density populations, producing litter sizes averaging 1.4  
93 cubs per annum (Annavi *et al.* 2014a). Pre-natal mortality has been estimated at 15-  
94 20% (Wandeler & Graf 1982), whilst failure to develop and implant blastocysts  
95 (Anderson & Trewhella 1985), loss of embryos (Page *et al.* 1994) through  
96 reabsorption (Kent & Webberly 2004) and abortion of litters (Page *et al.* 1994) are  
97 common. Studies on badger population dynamics typically discuss reproductive  
98 failure in view of environmental (Macdonald *et al.* 2009; McCallum *et al.* 2009;  
99 Nouvellet *et al.* 2013) or behavioral factors (Annavi *et al.* 2014b), and in terms of

genetic compatibility (Annavi *et al.* 2014a), disregarding potential involvement of STDs. Any research on diseases has been restricted to non-venereal infections (Newman & Byrne, in press).

Two HV have been reported in European badgers: An alpha-HV, tentatively classified as *Mustelid alphaherpesvirus 1* (MusAHV-1), was recently identified from lymph nodes, salivary glands and liver of French badgers (Sandrine Lesellier & Bernhard Ehlers, unpubl. data), and a gamma-HV, currently classified as *Mustelid gammaherpesvirus 1* (MusGHV-1). MusGHV-1 was originally detected in badger lung tissue (Banks *et al.* 2002) and has since been confirmed to be highly prevalent in the blood of badgers throughout the UK (King *et al.* 2004; Sin *et al.* 2014). The pathogenicity of HV in badgers, however, is unknown.

Here, we scan a well-studied high-density badger population for alpha-, beta-, and gamma-HV. As MusGHV-1 has been isolated from multiple body sites, and adverse health effects of related HV-infections are well documented in other species, we relate genital MusAHV-1 and MusGHV-1 infection status to life-history data to investigate potential correlation between HV-infection status and badger somatic fitness and/ or impaired female reproductive fitness. Considering that vertical transmission of HV is possible, we expect a proportion of cubs to be infected with MusAHV-1 and MusGHV-1 before reaching sexual maturity.

## **Materials and Methods**

### *Study population and sample collection*

122 The study was conducted in a high-density badger population ( $44.55 \pm 5.37(\text{SE})$   
123 badgers/ km: Macdonald *et al.* 2015) in Wytham Woods, a 424-ha mixed woodland  
124 in Oxfordshire, UK ( $51^{\circ}46'02''\text{N}$ ,  $1^{\circ}19'01''\text{W}$ : Savill *et al.* 2010).

125 Trapping was carried out over two weeks in May/June (spring, when cubs were fully  
126 weaned) 2015 as part of an ongoing long-term population study (Macdonald *et al.*  
127 2015). Badgers were caught in steel mesh box traps (85 x 37 x 38 mm) baited with  
128 ca. 150 g of peanuts and then transferred to holding cages and transported to a  
129 central holding facility, before being sedated by intramuscular injection of 0.2 ml  
130 ketamine hydrochloride/ kg body weight ('Ketamidor'; Chanelle, Hungerford,  
131 Berkshire, U.K: McLaren *et al.* 2005).

132 All trapping and handling procedures were carried out under Natural England license  
133 2014-5710-SCI-SCI, Home Office license PPL 30/2385, and approved by the  
134 University of Oxford's Zoology Ethical Review Committee. All procedures were in  
135 accordance with the ethical guidelines provided by the United Kingdom Animals  
136 (Scientific Procedures) Act, 1992, and the American Society of Mammalogists.

137 At first capture (usually as cubs), all badgers received a permanent unique tattoo in  
138 the left inguinal region, enabling individual identification and age classification as cub  
139 (< 1 year), yearling (< 2 years, sexually immature) or adult ( $\geq 2$  years: Tinnesand *et*  
140 *al.* 2015). Tattoo, sex and social group membership (determined by bait-marking:  
141 Buesching *et al.* 2016) were recorded, and body condition was categorized as 1 =  
142 emaciated to 5 = very good condition (Buesching *et al.* 2009). Reproductive status of  
143 adult females was deduced from vulva condition and classified as estrus (vulva  
144 swollen and moist), or non-estrus (vulva flat and dry: Tinnesand *et al.* 2015).

145 Reproductive success was inferred from whether a female had lactated as  
146 determined by teat size (Dugdale *et al.* 2011) and confirmed by genetic pedigree for

the year in which samples were collected to assess impact of current infection on cub survival ("current reproductive success"), and also for the following spring to assess impacts of infection on subsequent natality/ cub survival ("future reproductive success"). Clinical signs of infection, such as vulva/penile abnormalities or discharge were recorded and rectal temperature taken to screen for pyrexia. The genital mucosa of the vulva or penis was swabbed gently with pre-sterilised woodstick shaft cotton tip swabs (TS/6-H; Technical Services Consultants Ltd, Heywood, Lancashire, UK), carefully avoiding any contamination with blood (e.g. from suppurating infections), as confirmed by visual inspection. All samples were frozen immediately, and stored at -20°C until further analysis. After recovery from sedation ( $\geq 3$  h), all animals were released at their sett of capture.

#### *HV-Screening*

DNA was extracted from 98 genital swabs (71 females: 51 adults and 20 cubs; 27 males: 26 adults and 1 cub) as recommended by the manufacturer using QIAamp DNA Mini Kits (QIAGEN Ltd, Manchester, UK). Swabs were placed in 2 ml PBS containing Piramicin at  $5 \mu\text{g ml}^{-1}$  for 1 h. The swabs were removed and the samples spun at  $5,000 \times g$  for 5 min. To lyse the cell walls of Gram-positive bacteria, the pellet was re-suspended in 180  $\mu\text{l}$  of enzymatic lysis buffer containing 20  $\text{mg mL}^{-1}$  of lysozyme and 200  $\mu\text{g ml}^{-1}$  lysostaphin and incubated at 37 °C for 30 min in a shaking incubator. After adding 20  $\mu\text{l}$  of Proteinase K, samples were incubated at 57°C for 1 hour, followed by 15 min at 95 °C. The lysate was then purified according to the QIAamp kit protocol and all samples stored at -20°C.

DNA extracts were screened for the presence of alpha-, beta- or gamma-HV using a generic nested panherpes (panHV)-PCR analysis (Ehlers *et al.* 1999) for

amplification of 160 bp to 181 bp of the HV DPOL gene (primer-binding sites excluded). Primers were 285s DFA: 5'-GAYTTYGC[N/I]AGYYT[N/I]TAYCC-3', primer 285s ILK: 5'-TCCTGGACAAGCAGCAR[N/I]YSGC[N/I]MT[N/I]AA-3', and primer 285-as KG1: 5'-GTCTTGCTCACCAG[N/I]TC[N/I]AC[N/I]CCYTT-3' in the first PCR round, followed by primer 286s TGV 5'-TGTAACCTCGGTGTAYGG[N/I]TTYAC[N/I]GG[N/I]GT-3' and primer 286-as IYG: 5'-CACAGAGTCCGTRTC[N/I]CCRTA[N/I]AT-3') in the second round. The PCR reaction mix (25 µl) included: 1 µM of each PCR primer (Metabion, Martinsried, Germany), 200 µM of each deoxynucleotide triphosphate, 1 unit of DNA Polymerase AmpliTaq Gold and 2.5 µl GeneAmp 10 x PCR buffer with 2 µM MgCl<sub>2</sub> (Applied Biosystems GmbH, Darmstadt, Germany), and 5 % DMSO (Sigma-Aldrich Chemie GmbH). The reaction mixes in both PCR rounds were kept at 95° C for 12 min for polymerase activation, and then cycled 45 times with 20s of denaturation at 95° C, 30s of annealing at 46° C and 30s of strand extension at 72° C, followed by a final extension step at 72° C for 10 min. In all PCRs, 10 µl of genital swab DNA were used as template, and DNA extracts of MusAHV-1 and MusGHV-1 positive organs (Lesellier & Ehlers, unpubl. data) were used as positive controls. All products from the panHV PCR of expected length were purified and sequenced using the Big Dye terminator cycle sequence kit (Applied Biosystems, Warrington, UK) on a 377 automated DNA sequencer (Applied Biosystems, Warrington, UK).

To determine the detection limit of the panHV PCR for MusGHV-1 sequence, a MusGHV-1 PCR product was generated that spans all primer-binding sites of the panherpes PCR in MusGHV-1 DNA polymerase coding sequence (Genbank accession number: AF376034). This was then serially 10-fold diluted in HV-negative mammalian DNA (5 ng / PCR reaction). The dilution series (with 10.000 to 0.001



fragment copies per PCR reaction) was then tested with a pan-HV PCR. From this we estimated the detection limit of the pan-HV PCR for MusGHV-1 sequence to be 100 copies / PCR reaction.

Although the generic PCR used here has been shown to be able to detect alpha-, beta- and gamma-HV (e.g., Ehlers *et al.* 1999, 2007, 2008), here it detected only gammaHV. However, as the detection of the gamma-HV in so many samples might have masked the detection of any alpha- or beta-HV, we also tested specifically for MusAHV-1 (Genbank accession number: release of MF042164) using a nested PCR with the primers 7207-s 5'-GGTTTATTACCTTGTCTACACATAGCT-3' and 7207-as 5'-TTTACATAGTAAGATTTGTTTCGGAACG-3' in the first round (amplification product: 170 bp, without primer binding sites) and 7489-s 5'-ACCTTGTCTACACATAGCTGCA-3' and 7489-as 5'-GGAACGGCTATGGATGAGGC-3' in the second round (amplification product: 141 bp, without primer binding sites). PCR assays were run on a TgradientS thermocycler (Biometra, Göttingen, Germany) accordingly for the first round: 95°C for 12 min, 45 cycles of denaturation at 95°C for 20s, annealing at 62°C for 30s, elongation at 72°C for 2 min, and a final elongation at 72°C for 15 min; with an annealing temperature of 61°C in the second round. A DNA extract of a MusAHV-1 PCR-positive organ (Lesellier & Ehlers, unpubl. data) was used as positive control.

To estimate the detection limit of this PCR for MusAHV-1 sequence, a MusAHV1 PCR product was generated with the panHV-PCR that spans all primer-binding sites of the MusAHV1-specific PCR in MusAHV1 DNA polymerase coding sequence. It was serially 10-fold diluted in MusAHV1-negative mammalian DNA (5 ng / PCR reaction). MusAHV1-specific PCR was then performed with this dilution

series (10.000 to 0.001 fragment copies per PCR reaction). From this we estimated a detection limit of 100 MusAHV-1 copies / PCR reaction.

All statistical analyses were carried out using the statistical software in R. A Wilcoxon rank-sum test and Fisher's exact test were used to investigate potential effects of herpesvirus infection on body and reproductive condition respectively. To test for differences in current and future reproductive success based on infection status, a Chi-squared test with Yates continuity correction was employed.

## Results

### *Inter-individual differences in HV-infection*

All swabs tested negative for alpha- and beta-HV, both in the pan-HV generic PCR as well as in the MusAHV-1 specific PCR. However, of the samples tested with panHV generic PCR (n = 98), a total of 54 swabs (39 adults, 15 cubs; i.e. 55 %) tested positive for a gamma-HV, determined as MusGHV-1 by sequencing.

Both, MusGHV-1 infected and uninfected badgers were detected during the same trapping event in 83% (15/18) of the social groups, where more than 1 badger (male, female or cub) was tested. In only 11% (2/18) of groups all individuals (n=4; 3 adult females and 1 adult male in each) tested were positive, and in only 6% (1/18) both badgers (n=2; 1 adult female and 1 adult male) tested in this group were negative, indicating wide-spread venereal MusGHV-1 infection in this population. Furthermore, in 44% (8/18) of these social groups displaying heterogeneous infection, cubs were also infected (n=12 females), inferring vertical transmission of MusGHV-1.

## *Fitness-consequences of HV-infection*

MusGHV-1 infection had no effect on female (Wilcoxon rank-sum test:  $W = 144.5$ ,  $p > 0.05$ ) or male body condition (Wilcoxon rank-sum test:  $W = 144.5$ ,  $p > 0.05$ ).

Infection had no effect on female reproductive condition (Fisher's exact test,  $p = 1$ ), with near equal ratios of infected (7/22 = 32%) and uninfected females (9/29 = 31%) being in estrus, compared to non-estrus (infected: 15/22 = 68%; uninfected: 20/29 = 69%), and current female reproductive success did not relate to infection status (Chi-squared test with Yates continuity correction:  $X^2 = 0.005$ ,  $df = 1$ ,  $p = 0.94$ ). Future reproductive success, however, tended to be lower in infected females with only 20% of infected females (3/15) displaying signs of recent lactation the following year, compared to 50% (7/14) of uninfected females, although this trend was not significant due to small sample sizes (Chi-squared test with Yates continuity correction:  $X^2 = 1.709$ ,  $df = 1$ ,  $p = 0.19$ ).

None of the infected individuals (i.e., neither males nor females) displayed clinical signs of infection, fever or genital discharge.

## **Discussion**

This is the first systematic investigation of the occurrence and prevalence of HV in the genital tract of any wild terrestrial carnivore. We did not detect any alpha- or beta-HV. Although the panHV PCR may not detect all vertebrate HV with equal sensitivity, this technique has been shown to discover novel alpha-, beta-, and gamma-HV in a wide range of host species including carnivorans (e.g., Ehlers *et al.* 1999, 2007, 2008), and served to identify MusAHV-1 in badger organs (Lesellier & Ehlers, unpubl. data). We are therefore confident that the lack of detection of alpha-

and beta-HV in genital swabs reported in this study was due to the absence of such viruses (at least in clinically relevant numbers) in the investigated samples.

We did identify, however, the gamma-HV MusGHV-1 in 43% of vaginal swabs from adult females, 65% of penal swabs from adult males and 71% of cubs (all female).

We posit that badger cub infection is likely to follow a vertical transmission route as established for various gamma-HV in other mammals (human HV-8: Pica and Volpi, 2007; ovine HV-2 in sheep: Baxter *et al.* 1997; otarine HV-1 in sea lions: Buckles *et al.* 2007).

In addition, the presence of MusGHV-1 in the genital tract of adult males and females indicates a plausible additional sexual transmission route as inferred for gamma-HV found in other species (e.g. Blainville's beaked whale, *Mesoplodon densirostris*: Saliki *et al.* 2006; California sea lions, *Zalophus californianus*: Lipscomb *et al.* 2000; mice, *Mus musculus*: Francois *et al.* 2013). Indeed, the promiscuous, polygynandrous mating system of badgers, whereby both males and females mate with multiple partners (Dugdale *et al.* 2007; Annavi *et al.* 2014a), would facilitate the spread of venereal HV within a population.

#### *Inter-individual variation in genital HV-prevalence*

The occurrence of genital HV-positive and negative individuals in the same social group indicates that some individuals are more susceptible to infection than are others, where genetic, behavioral and physiological factors could be accountable (Van de Laar *et al.* 1998). Promiscuity is typically reflected in high prevalence and virulence of STDs (Kokko *et al.*, 2002; people: Jonsson & Warren 2004), and, in their presence, female mating strategies can diversify (Kokko *et al.* 2002). Thus, both, 'risky' females (i.e., those that mate indiscriminately), and 'risk-averse' (i.e., choosy)

294 females can persist in a population as long as risky behavior accrues genetic  
295 benefits (Boots & Knell 2002). Indeed, although badgers are induced ovulators, and  
296 thus require multiple matings for successful fertilization (Yamaguchi *et al.* 2006), and  
297 delayed implantation coupled with superfecundation and superfetation increases  
298 genetic diversity through multiple paternity within the same litter (Annavi *et al.*  
299 2014a), some females appear to be more choosy than others and have been shown  
300 to reject certain males (Woodroffe & Macdonald 1995). Nevertheless, explanations  
301 of female mate choice on the basis of genetic diversity and offspring survival benefits  
302 remain tenuous (Annavi *et al.* 2014a; Sin *et al.* 2015). In general, high levels of gene  
303 diversity in the Major Histocompatibility Complex (MHC) are linked to pathogen  
304 resistance (people: Hughes & Nei 1992; mice, *Mus musculus*: Penn 2002; domestic  
305 dog, *Canis lupus familiaris*: Quinnell *et al.* 2004), whereas badgers typically mate  
306 with individuals of similar MHC profiles (Sin *et al.* 2015). The resulting reduction in  
307 MHC heterozygosity, however, is not correlated with intensity of hematological  
308 MusGHV-1 infection (Sin *et al.* 2015). Instead, carriers of allele *Meme DRB\*01*  
309 exhibit higher intensity of hematological infection, whilst carriers of allele *Meme-*  
310 *MHCI\*03* have lower infection intensity (Sin *et al.* 2015). Thus, we posit that more  
311 'choosy', risk-averse females may gain immune-advantages for their offspring in  
312 favour of genetic diversity (Nowak *et al.* 1992) while simultaneously minimizing their  
313 own exposure to STDs. Females that possess the *Meme-MHCI\*03 allele* may be  
314 more resistant to genital MusGHV-1 and could thus afford to follow a more 'risky'  
315 strategy. If indeed MusGHV-1 is sexually transmitted, the variability in male infection  
316 rate seen across social groups in this study could also be attributed to mating  
317 strategy. Males seeking extra-pair paternity to increase their fitness through siring  
318 more offspring would naturally be more exposed to infection than those limiting their

mating opportunities. In this study population, >40% of litters result from extra-group matings (Dugdale *et al.* 2007). Diverse promiscuity has been observed in both sexes (Annabi 2014a) and therefore offers plausible explanation for the observed heterogeneity of MusGHV1 infection also in males.

#### *Potential fitness effects of genital HV*

HV typically suppress immune responses, which in turn can trigger concomitant infections with other diseases (Widén *et al.* 2012) and cause oxidative stress (Kavouras *et al.* 2007), leading to damage of the reproductive tract and subsequent infertility (Tremellen 2008) as well as reactivation of latent (HV-) infections (cattle: Wentink *et al.* 1993; squirrel monkeys, *Saimiri sciureus*: Varnell *et al.* 1995; people: Ye *et al.* 2011). Previous blood analyses confirmed near 100% systemic MusGHV-1 infection rates of badgers throughout the UK and Ireland (98% in this study population (Sin *et al.* 2015); southwest England: 95%, Ireland: 100%: King *et al.* 2004), compared to the 55% infection rate with genital HV found in this study. Nevertheless, although infection intensity of hematological MusGHV-1 is correlated negatively with body condition (Sin *et al.* 2015), in our study, genital gamma-HV infection was not linked to somatic fitness in either sex.

Reproductive failure is common in badgers, with up to 80% of females failing to implant and approximately 38% absorption rates of fetuses (Cresswell *et al.* 1992). Previous research has linked these to low body condition (Cresswell *et al.* 1992; Woodroffe & Macdonald 1995) and/ or speculated on underlying behavioral mechanisms of reproductive suppression (Page *et al.* 1994; Woodroffe & Macdonald 1995), but has not considered the potential involvement of genital HV or any other

STDs. We found no evidence of genital gamma-HV infection being correlated with previous reproductive success, but there was a trend (albeit not significant, plausibly due to small sample sizes) that could indicate that genital MusGHV-1 might decrease chances of completing concurrent pregnancies to full term and/or bear viable offspring. Gamma-HV express specificity for either B or T lymphocytes and establish long-term latency, most probably in lymphoid tissue with intermittent reactivation of HV due to environmental stressors (Widén *et al.* 2012). They then typically infect mucosa and epithelial tissue (e.g. Goldstein *et al.* 2004; Widén *et al.* 2012; Cabello *et al.* 2013). It is important to consider therefore that the potential negative impact on reproductive success observed in this study could also result from other immune stressors such as oxidative stress and/or a primary disease (e.g., bTB) established in the host, which often precedes HV infections (Winkler *et al.* 1999; Tryland *et al.* 2009).

Similarly, the sporadic infections spread across social groups in our study population and seasonal differences between MusGHV-1 infection in the blood and genital tract could also be attributed to oxidative stress, and thus reflect variability in badger antioxidant capacity, particularly during the mating season. In addition, infections with members of the *Alphaherpesvirinae*, which typically incite genital lesions, abortion, stillbirth, foetal resorption and neonatal death in domestic and wildlife species (Smith 1997; Widén *et al.* 2012), can remain latent for life with sporadic recrudescence (Widén *et al.* 2012). Thus, the absence of alpha-HV in our genital swab samples does not exclude the possibility of latent infections with MusAHV-1 or other alpha-HV (Widén *et al.* 2012; das Neves *et al.* 2009).

## Conclusion

The results of this study indicate that the highly promiscuous mating system of badgers most likely leads to genital MusGHV-1 infection at the population-level, as seen for hematological infection (Sin *et al.* 2015). Despite almost all individuals acting as carriers of the virus (Sin *et al.* 2015), individual differences in their reproductive strategies, immune-functioning and genetics could explain observed inter-individual variability in genital HV-prevalence. Because reproductive failure in badgers can be due to a multitude of reasons (Macdonald *et al.* 2015), the underlying causative factors for our current result need further investigation. As we observed no clinical signs of HV-infection, such as discharge, lowered body condition or fever (King *et al.* 2004; Dandár *et al.* 2010), this presents important implications for the screening of HV-infections in wildlife: If prevalence is not to be under-estimated, ‘apparently healthy’ individuals also need to be tested.

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