Routes for the potential transmission of bovine tuberculosis in the farmed environment

Submitted by

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Abstract:

Pathogens which can infect wildlife, domestic species and humans can have serious health, welfare and economic implications around the world. Bovine tuberculosis (bTB), caused by *Mycobacterium bovis*, is a widespread disease that affects a wide variety of livestock, wild mammals and humans. In England bTB remains a substantial problem, despite long term management strategies. It is estimated to cost £120 million per year and in the year ending September 2020, 27,339 test-positive cattlewere slaughtered. *Mycobacterium bovis* is proving difficult to manage, in part due to its generalist nature, infecting a broad variety of wild mammal species, and its ability to survive for extended periods of time in environmental substrates including water, soil and faeces. In this thesis, I explore two potentialsources of *M. bovis* in the environment: water troughs, and the spreading of cattle manure and slurry onto the farming landscape. Using both camera traps and GPS collaring technology, I find that wild mammals do not regularly drink from water troughs, suggesting that the role of water troughs in interspecific transmission might be limited, although their potential role in intraspecific transmission warrants further investigation. By attaching GPS trackers to both muck spreaders and slurry tankers to monitor their movements, I find that slurry and manure spreading by farmers does not change in response to the detectionof test-positive cattle on their farms, providing the opportunity for pathogen spread (both on and off the home farm). Whilst both water and cattle faeces management are covered in advisory guidance, their management to prevent infection risks is not enforceable. bTB provides an example of a disease with multiple possible routes of interspecific and intraspecific transmission, and for successful rapid disease control an understanding of all routes is necessary.

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Author's declaration

All chapters in this thesis were written by Kelly Astley with comments provided by Professor Rosie Woodroffe and Professor Robbie McDonald (my supervisors).

Chapter 2: Camera trap data collection was carried out by Kelly Astley and Cally Ham. Water trough samples were collected by Kelly Astley and Seth Jackson and qPCR on these samples was carried out by University of Warwick and results used with their permission. Badger GPS collaring was undertaken as part of a long-term monitoring project led by Professor Rosie Woodroffe.

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Chapter 1: General introduction

Wildlife/livestock Disease

In recent times, wildlife diseases have received increasing amounts of public attention, especially those which constitute a human and livestock health concern or incur a financial burden (Kruse *et al*., 2004). It has been estimated that 58% of all human pathogens originated in animals and 72% of diseases that are increasing in incidence originate in wildlife (Woolhouse and Gowtage-Sequeria, 2005; Jones*et al.*, 2008). Pathogens which can infect wildlife, domestic species and humans can have serious health, welfare and economic implications around the world. For example, avian influenza A (H5N1) virus is a disease of domestic poultry and wild birds, which can also infect a range of mammalian species including humans, with often fatal consequences; this virus constitutes a potential pandemic threat in humans and has taken a huge toll on the poultry industry in many developing countries (Peiris *et al.,* 2007).

The threat of wildlife diseases is becoming more frequent due to an increase in anthropogenic factors that are aiding disease transmission between human, wildlife and livestock populations (Woolhouse and Gowtage-Sequeria, 2005; Jones *et al.*, 2008; Borzée *et al.*, 2020). Conversion of natural habitat to agricultural land creates opportunities for transmission of pathogens between wildlife, livestock and humans (Dobson and Foufopoulos, 2001). A recent review found that people working in recently cleared, rural areas in southeast Asia were 1.74 (CI:1.47 – 2.07) times more likely to be infected with a pathogen(Shah *et al.,* 2019). Additionally, fragmentation of woodland and conversion to grassland has been associated with an increase in ixodid ticks, carriers of *Borrelia* spp., associated with Lyme disease, which can cause disease in both humans and livestock (Li *et al.,* 2012; Parker and White, 1992)

Wildlife Disease Management

The main aim of wildlife disease management is to reduce pathogen transmission between wildlife and humans and/or domestic livestock with the aim to prevent, control or eliminate disease. Management strategies vary depending on factors including the species involved and transmission routes, and have over time shown varying degrees of success. In Africa, buffalo (*Syncerus caffer*) can become infected with foot-and-mouth disease (FMD) and act as a primary carrier. Outbreaks of FMD in cattle have severe health and economic consequences for farmers (Maree *et al.*, 2016). Fencing has been used successfully to reduce prevalence of FMD on very large scales, however it is expensive to install and maintain, and can cause unintended issues for other species, for example, such large barriers (some over 500km) can interfere with large scale animal migration (Gortazar *et al.*, 2015).

Management of pathogen transmission by separation of wildlife and livestock can also be achieved through selectively grazing areas where wildlife is not present.

For example, malignant catarrhal fever can be passed by wildebeest tocattle, with devastating consequences for Masai cattle herds. This risk can be somewhat managed by grazing cattle away from wildebeest calving grounds (Russell, *et al.,* 2009). However, this comes at a socio-economic and environmental cost, as wildebeest calving grounds are preferentially sold to be converted to arable land, and cattle are moved further from enclosures, resulting in less milk being available to human householders, particularly the elderly, women and children (Lankester *et al.*, 2015).

Where separation of wildlife and livestock is deemed difficult, inappropriate or impossible, wildlife culling may be used in an attempt to reduce opportunities for disease transmission (Gortazar *et al.*, 2015). For example, the common vampire bat (*Desmodus rotundus*) feeds on mammalian blood, providing a mechanism of rabies virus transmission to both livestock and humans, and many Latin American countries cull vampire bats to reduce population size with the aim of reducing opportunities for pathogen transmission (Becker *et al.,* 2015). Similarly, in the USA non-native European starlings (*Sturnus vulgaris*) have been culled due to their association with transmission of *Salmonella enterica* between cattle herds, a method that has had some success (Carlson *et al.*, 2011).

For disease management to be effective it is important to have a clear understanding of both disease ecology and animal behaviour (McDonald *et al.,* 2018). The desired effect is not always achieved in complex systems and detrimental impacts may occur. For example, in an attempt to limit transmission of *Brucella abortus* on shared pastures, elk (*Cervus canadensis)* were subject to massive supplementary feeding in areas where they would not come into contact with cattle; this caused large aggregations of elk and increased prevalence of infection, therefore potentially increasing future risk to grazing cattle (Brennan *et al.,* 2014; Cross *et al.,* 2007). The effect of control measures on host behaviour may also influence the outcome of management interventions. Red foxes (*Vulpes vulpes*) and raccoon dogs (*Nyctereutes procyonoides*) were culled across mainland Europe in an attempt to control rabies; the subsequent population reduction resulted in increased dispersal and re-establishment of territorial boundaries through fighting and this aggression increased contact opportunities between individuals and raised transmission rates (Holmala and Kauhala, 2006). A more comprehensive understanding of disease ecology and animal behaviour would help to predict when managementprogrammes may cause increased risk of the very disease they are trying to control.

Establishing appropriate surveillance schemes is of the upmost importance in wildlife disease, even if active management is not required (Gortazar *et al.*, 2015). Prompt detection of new and re-emergent diseases allows counter measures to be implemented quickly, consequently lessening the potential impact of the disease (Morner *et al.*, 2002). Since 2006, dead wild birds foundin Europe can be submitted for testing for avian influenza, which can be transmitted to farmed birds and people. This surveillance has enabled rapid detection of potential virus hotspots and improved knowledge about which wild bird species are most likely to be susceptible to disease (Hesterberg *et al.*,2009; Adlhoch *et al.*, 2020). Surveillance schemes, whilst not always practicalor possible, can provide vital information to inform disease management.

Bovine Tuberculosis

Bovine tuberculosis (bTB), caused by *Mycobacterium bovis*, is a widespread disease that affects a wide variety of livestock, wild mammals and humans (O'Reilly & Daborn 1995; Delahay *et al.,* 2007). Problems posed by bTB arise predominantly from infections of humans and farmed cattle. Historically high levels of human infection with *M. bovis* were due to consumption of infectedmilk and in the UK the decline in human infections was brought about after the introduction of pasteurisation in the early twentieth century (De La Rua- Domenech, 2006). Currently, in Africa, human infection with *M. bovis* (from consumption of infected cattle products) is associated with further complicationsof the HIV/AIDS pandemic and is a severe health risk to immunocompromised people (Ayele *et al.,* 2004).

M. bovis excreted into the environment through urine, faeces or sputum may persist for an extended period of time (Courtenay *et al*., 2006; Fine *et al*., 2011), potentially adding to mechanisms of transmission. *M. bovis* bacilli are able to withstand dehydration, change in temperature, effects of sunlight and changesin pH (Duffield and Young, 1984; Rodríguez-Hernández *et al*., 2016). Viable *M. bovis* bacilli have been found up to four months after initial environmentalcontamination, and *M. bovis* DNA has been detected for up to 21 months(Williams & Hoy, 1930; Young *et al*., 2005). The presence of *M. bovis* in the environment, and its persistence, provides the potential for transmission between cattle, wildlife, and humans (Biet *et al.,* 2005; Courtenay *et al.,* 2006; Fine *et al.,* 2011).

Due to the risk posed to human health, infection in cattle herds is managed through the use of test and slaughter programmes. In the UK the predominant test that is currently used is the single intradermal comparative cervical tuberculin (SICCT) test. With this test, individual cattle are injected sub dermallywith two proteins, one derived from *M. bovis* and one derived from *M. avium*, After 72 hours, the two injection sites are compared; if the inflammatory response is substantially greater at the site of *M. bovis* than *M. avium* a positive result is given (Monaghan *et al*., 1994). Farms are given officially TB Free(OTF) Status when they have no test positive cattle, this enables them to move and sell cattle without restriction. If a herd has a positive test result, it isclassified as OTF - suspended (OTFS) and if after slaughter the animal is foundto have lesions characteristic of bTB, or a laboratory culture confirms infection with *M. bovis*, then the herd becomes OTF – withdrawn (OTFW). Once a herd has had a confirmed test positive individual (reactor) its cattle are placed under movement restrictions (AHVLA, 2014; DEFRA, 2014b) that severely constrain movement of cattle off the affected farm.

As *M. bovis* is a generalist pathogen, found in a variety of wildlife species (Delahay *et al*., 2007; Réveillaud *et al*., 2018), control of infection within nationalcattle herds has been supplemented with population control of known wildlife host species. Between 1970 and 1997, Australia eliminated bTB from cattle using an intensive management programme, focused on test and slaughter of infected cattle and the culling of both feral pig (*Sus scrofa)* and water buffalo *(Bubalus bubalis)*, both of which were known to have high prevalences of *M. bovis* infection (Corner, 2006).

In New Zealand, restrictions of cattle movementsfrom herds with test-positive individuals and simultaneous removal of invasive brushtail possums (*Trichosurus vulpecula)*, greatly reduced cattle *M. bovis* infection from 3.8% of mature cattle infected in 1994 to <0.003% in 2013(Livingstone *et al.,* 2015; Ryan *et al.,* 2006). In the USA, *M. bovis* infection is primarily confined to Michigan state, where infection is present in both cattleand white-tailed deer (*Odocoileus virginianus)*; successful reduction of disease prevalence has been achieved in both populations by reducing deer numbers (increasing hunting permits) and reducing deer-to-cattle contact, by excluding deer from over winter feeding stations (O'Brien *et al.,* 2006; Schmitt *et al.,*2002). Although bTB in cattle is widespread in Africa, prevalence and incidence of *M. bovis* is chronically under reported. Infection has been found in a diverse range of wildlife, including, warthogs (*Phacochoerus aethiopicus*), Cape buffalo (*Syncerus caffer*), baboons (*Papio ursinus*) and cheetahs (*Acinonyx jubatus*). In mainland Europe *M. bovis* infections persist in several countries, many wildlife species have been found with varying prevalence of *M. bovis* infection (including, red deer (*Cervus elaphus*), fallow deer (*Dama dama*), red foxes (*Vulpes vulpes*) and wild boar (*Sus scrofa*). In Spain, attempts to reduce *M. bovis* infection through culling wild boar have had mixed results (Boadella *et al.,*2012; García-Jiménez *et al.,* 2013).

Within the UK, control of bTB is a devolved issue, under control separately by the governments of England, Wales, Scotland and Northern Ireland. In England bTB has been termed "the most pressing animal health problem" (DEFRA2014a) and is an endemic disease, incurring estimated costs of £120 million peryear (Godfray *et al.,* 2018). The Government invests heavily in payments for cattle testing, compensation for slaughtered stock and research into methods of reducing infection, while farmers bear additional financial burdens including, lostearnings from being unable to sell movement restricted cattle and additional feed and housing required to keep unsellable livestock (DEFRA, 2014b; Godfray *et al.,* 2018). Despite cattle bTB controls being in place since the1930s, the disease is currently far from being eliminated, the number andgeographical extent of cattle herds infected with *M. bovis* has increased sinceits nadir in the 1970s and has only recently shown signs of decline (DEFRA, 2020a).

In the UK, *M. bovis* infection has been found in the following wildlife; badgers (*Meles meles*), fallow deer (*Dama dama*), sika deer (*Cervus nippon*), red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), red fox, ferret (*Mustela furo*), mink (*Neovison vison*), feral cat (*Felis catus*), mole (*Talpa europaea*), brown rat (*Rattus norvegicus*) and wild boar (Delahay *et al.,* 2001; Ward *et al.*, 2009; Broughan *et al.*, 2013). Of these species, only badgers have been proven to play a large-scale role in wildlife to cattle transmission of *M. bovis* in the U.K. (Donnelly & Nouvellet, 2013), although the presence of infections in deer populations may pose considerable localised risks (Crispell *et al*., 2020; Ward *et al*., 2009). Badgers were first implicated in the transmission of *M. bovis* in 1971 when a dead badger, found in Gloucestershire, was found to be infected (Muirhead *et al.,*1974). Badgers are now thought to potentially contribute to the maintenance of bTB in cattle (Krebs *et al.*, 1997; Bourne, 2007; Biek *et al.*, 2012). Within areas of high bTB incidence, an estimated 5.7% (95% CI: 0.9- 25%) of transmission into cattle herds is due to badger-to-cattle transmission (Donnelly & Nouvellet, 2013).

The route of interspecific transmission of *M. bovis* is not fully understood.Studies have found that direct badger to cattle contacts are rare (Drewe *et al.,* 2013; Woodroffe *et al.,* 2016), suggesting that indirect transmission via a shared environment is the most likely mechanism for transmission. The location of indirect contact events is most likely to be at pasture, where cattle graze and badgers forage (Woodroffe *et al.*, 2016). Pasture fields present the opportunity for both badger to cattle transmission of *M. bovis*, through cattle encountering infectious badger urine or faeces and the opportunity for cattle to badger transmission through badgers foraging in areas contaminated with infectious cattle excreta (Böhm *et al.*, 2008; Woodroffe *et al.*, 2016). Understanding of when and where indirect contact events occur is important for modelling disease transmission and in designing mitigation measures. Specific locations within the farm environment identified as potentially posing a risk to cattle or wildlife include cattle feed troughs, cattle water troughs, mineral licks, barns, badger latrines and setts accessible to grazing cattle (Garnett, *et al.,* 2002; Tolhurst *et al.*, 2009; Woodroffe *et al.*, 2017; Robertson *et al.*, 2019). Monitoring of these features to determine if *M. bovis* is present and in what quantities, alongside monitoring of how they are used by different species is important for designing targeted management plans.

Aims

Within this thesis, I explore the potential roles that water troughs (Chapter Two), and the spreading of cattle faeces (Chapter Three) might play in the interspecific and intraspecific transmission of *M. bovis* in the farming environment. To address these questions, I used GPS tracking of both badgers and of farming equipment alongside remote camera trapping to characterise interactions with potential sources of infection. Such information is useful in helping to understand the disease dynamics on cattle farms in England and in designing management methods to reduce opportunities for infection within the cattle population.

Chapter 2:

The potential role of water troughs as locations for transmission of *Mycobacterium bovis*.

Abstract

Fomites are important locations for the transmission of environmentally persistent pathogens. Understanding encounters with fomites on the part of domestic and wild animals might enable reduction of risks of transmission and aid disease control. *Mycobacterium bovis,* the causative agent of bovine tuberculosis (bTB), is persistent in the environment and such persistence is a major cause of concern for disease transmission, both among cattle, and between cattle and wildlife. Transmission from badgers (*Meles meles*) is known to contribute to the maintenance of bTB in cattle, and while direct transmission between badgers and cattle appears relatively unlikely, indirect transmission via contamination of shared environments appears to be the most probable route oftransmission between these species. *M. bovis* has been shown to survivereadily in water and so opportunities for disease transmission may be most apparent at permanent water sources. We monitored use of 13 cattle water troughs on five farms, using remote cameras. The troughs were also subject to testing for *M. bovis*. Over 305 trough-days of monitoring, we detected 3,238 individual drinking events for sheep, 1,151 for multiple species of wild birds, 214for cattle and two for badgers. Sheep and wild birds made significantly more visits to *M. bovis* test-positive troughs (n = 6) than to test-negative troughs (n = 7). The infrequent use of water troughs by badgers was confirmed using GPS- collar tracking, which showed that 68 collared badgers avoided using space ≤5m from water trough locations. The infrequent use of water troughs by badgers suggests that in our study area water

troughs were unlikely to play a major role in *M. bovis* transmission between badgers and cattle. Our results do not, however, exclude the possibility that water troughs may contribute to other *M. bovis* transmission routes.

Introduction

Fomites are important locations for transmission of pathogens that persist well in the environment (Kraay *et al.*, 2018). Infectious environmental fomites can provide opportunities for indirect transmission both within and between species, including between domestic animals and wildlife (Allen *et al.,* 2021). If disease elimination is to be achieved, control measures should target transmissions of multi host pathogens, as transmission between domestic animals and wildlife can occur in both directions (Dobson and Foufopoulos, 2001). Understanding encounters with environmental fomites by both domestic animals and wildlife might therefore aid the formulation of appropriate control strategies to minimise indirect transmission of infections.

Opportunities for interspecific transmission of infection have been shown to occur through water sources. Water-borne transmission has been shown to occur in avian influenza A viruses through sharing a contaminated water source; these viruses are able to persist in water for up to 60 days (Domanska- blicharz *et al.,* 2019; Rohani *et al.,* 2009; Wang *et al.,* 2012). The geographical distribution of leptospirosis, an acute bacterial disease that affects both humansand animals, is mediated by water-borne transmission; disease outbreaks are restricted to a few sites in the absence of flooding but pathogen contamination spreads to large areas during flooding events and the number of cases increases drastically (Codeço *et al.,* 2008).

Persistence in the environment of *Mycobacterium bovis,* the causative agent of bovine tuberculosis (bTB), is a cause of concern for transmission of the pathogen to both livestock and wildlife (Courtenay *et al*., 2006; Fine *et al.*,2011). *M. bovis* bacilli are able to withstand dehydration, change in temperature, effects of sunlight and changes in pH (Duffield and Young, 1984; Rodríguez-Hernández *et al.*, 2016). Viable *M. bovis* bacilli have been found up to four months after initial environmental contamination and *M. bovis* DNA has been detected for up to 21 months (Williams & Hoy, 1930; Young *et al.*, 2005).

In England, bTB is an endemic disease of cattle that imposes a great financial burden; it is estimated that £120 million per year is spent by farmers and taxpayers tackling bTB in cattle (Godfray *et al*., 2018). Despite bTB controls being in place since the 1930s the disease is far from being eliminated in the UK, the number and geographical extent of cattle herds in England and Wales infected with *M. bovis* has increased since the 1970s and has only recently shown signs of decline (DEFRA, 2020a). The current bTB control strategy in England involves routine testing of cattle herds; test positive animals are removed and slaughtered, and restrictions are imposed on herd movementsand trading, until the herd tests negative (AHVLA 2014; DEFRA, 2014). However, *M. bovis* bacteria can be shed by cattle in urine, faeces and respiratory tract secretions (Neill *et al.,*1994), and any *M. bovis* bacteria excreted by infected cattle may persist in the farm environment, even after the infected individuals are removed.

Direct cattle to cattle transmission constitutes the greatest transmission risk in bTB disease dynamics in cattle (Crispell *et al.,* 2019; Donnelly & Nouvellet, 2013). Although environmental contamination is thought to be a less effective route of disease transmission than direct contact (Menzies and Neill, 2000), the possibility of indirect transmission from cattle to cattle via the environmentshould not be overlooked. (Woodroffe *et al.*, 2016; Allen *et al.,* 2021).

Populations of badgers *Meles meles* are known to contribute to the maintenance of bTB in (Biek *et al.,* 2012; Bourne, 2007; Crispell *et al.,* 2019; Donnelly & Nouvellet, 2013; Krebs *et al.,* 1997). Within areas of high bTB incidence, 52% of cattle herd incidents are ultimately attributed to badgers, though only an estimated 5.7% (95% CI: 0.9-25%) of transmission eventsamong cattle arise proximately from badger-to-cattle transmission (Donnelly & Nouvellet, 2013). Studies have shown that transmission via direct contact between badgers and cattle is very unlikely (Drewe *et al.*, 2013; Woodroffe *etal.*, 2016), leaving indirect transmission via contaminated fomites in sharedenvironments as the most probable route of infection.

M. bovis has been shown to survive readily in water; *M. bovis* bacilli have been recovered from water exposed to natural weather conditions up to 58 days after experimental contamination (Fine *et al.*, 2011). In Spain, where wild boar have been shown to transmit *M. bovis* to cattle, opportunities for disease transmission are highest at permanent water sources (Barasona *et al.*, 2014).

Water troughs are provided for cattle, but may also be used by wildlife, including badgers. Investigations into badger use of water troughs have been conducted in Gloucestershire, Republic of Ireland and Northern Ireland (Campbell *et al.,* 2019; Garnett *et al.,* 2003a; Hahesy *et al.,* 1998; O'Mahony, 2014). Badger density varies, in relation to habitat type, across the UK and Ireland (Judge *et al.,* 2014; Reid *et al.,* 2008) and so further investigation into water trough use bybadgers across a range of densities and disease prevalences is warranted. We explored the potential role of water troughs in *M. bovis* transmission by using camera traps to compare wildlife and livestock use of water troughs, by testing troughs for *M. bovis*, and by characterising badger space use using GPS-collar tracking in four areas of Cornwall, where bTB incidence in cattle herds is high.

Methods

All data were collected between 2014 and 2018 from 20 farms distributedamong four study sites in Cornwall, South West England. Two of the sites were inland locations situated in north (C4) and east (C2) Cornwall, consisting of areas of mixed livestock (cattle and sheep) grazing and arable farmland. A third site (F1) was a coastal site in west Cornwall, consisting of pasture and grazed scrubland. The fourth site (F2) was at a coastal location in south west Cornwall, predominantly containing cattle grazing alongside arable farmland. All datawere collected with landowner consent. The badger density for each site has been previously estimated using the Minimum Number Alive method as $4.2.\text{km}^{-2}$at site C2, $5.5.\text{km}^{-2}$ at site C4 and $6.3.\text{km}^{-2}$ at both sites F1 and F2 (Woodroffe*et al.*, 2017).

The use of water troughs by livestock and wildlife was explored using camera traps. At all four study sites, trough water had been tested for *M. bovis* for a parallel project, using the qPCR method described in King *et al.* (2015). The test used is highly specific to *M. bovis*, therefore discounting other species within the *M. tuberculosis* complex*.* All troughs were tested four times in the preceding two years. For this analysis troughs were classified as positive when a trough had a minimum of one positive test result during that time. To ensure similar monitoring of test-positive and test-negative troughs, a team member not involved in the field data collection selected for camera trapping, an equal number of test-positive and test- negative troughs, from the same farms where possible. The troughs chosen for camera surveillance were located in three of the four study sites (C4, F1 and F2). Results of trough testing for *M. bovis* were not revealed to the field teams until fieldwork and analyses were completed.

A remotely operated trail camera ("camera trap"; Bushnell Trophy Cam or Bushnell Natureview) was secured to a fixed object close to each selected water trough, such that a picture would be taken when the water trough was used. Camera trap monitoring took place between April and June 2018. The cameras were checked regularly but due to camera failure monitoring days were not always consecutive. The resulting images were visually inspected, recording for each camera trap the number of days of monitoring, and the number of drinking events for each species observed. Birds were recorded as a group as *M. bovis* infection has notbeen found in wild bird species in the UK. A drinking event was recorded when an animal was seen ingesting water. If a drinking event was recorded over several sequential images this was counted as a single event.

Poisson regressions were used to compare the numbers of drinking events at *M. bovis* test-positive and test-negative troughs. These models included the fixed effects of farm identity, to account for uneven distribution of troughs and infection among farms and because species did not have equal access to all troughs, and the (natural log-transformed) numbers of days of monitoring for each camera trap.

As well as looking at use of water troughs using camera traps, we explored the use of the area around water troughs by badgers using GPS tracking. This approach allowed us to explore badger behaviour year-round, in contrast with the camera-trapping study, which had more limited duration. Badger movements were monitored at all four sites, as described in Woodroffe *et al.* (2016a). Badgers were trapped and handled under licence from the UK Home Office (project licence 70/7482) and Natural England (20122772) and following ethical review by the Zoological Society of London (projects BPE/0631 and PWE/691). Badgers were captured in wire mesh cage traps baited with peanutsin accordance with the above licences. Badgers were anaesthetised (De Leeuw*et al.,* 2004) and within each social group, at least one adult badger was fitted with a collar equipped with a GPS tag (Telemetry Solutions, Concord, CA,USA). The GPS collars were programmed to record a location every 20 minutesbetween 1800h-0600h GMT, unless the badger was classified as inactive by an on-board accelerometer. Data were collected regularly from the collars by remote download to safeguard against loss of data in case of collar failure. Datawere also downloaded when the collar was removed during subsequent trapping events. Efforts were made to remove collars from all badgers at theend of the study.

Potentially inaccurate GPS locations were removed by deleting locations recorded with fewer than four satellites or with a horizontal dilution of precision

(HDOP) that was greater than four (Woodroffe *et al.*, 2016). Previous analysis of the same dataset have shown that estimates of badger habitat selection, building use, and ranging behaviour were not altered by this filtering process (Ham, *et al.,* 2019; Woodroffe *et al.,* 2016, 2017a, 2017b). After filtering, GPS locations were shown on average to be accurate to 4.7 m (95% CI 4.5-4.9 m) (Woodroffe *et al.,* 2021).

Locations of all permanent water troughs were recorded using a handheld GPS unit during surveys of the study sites. Of the 421 water troughs recorded, 417 (99.1%) were located on field boundaries. Badgers have previously been shown to prefer to forage along field boundaries, this is thought to be because of greater abundance of invertebrate prey and/or favourable microclimate (Amy *et al.,* 2015; Facey *et al.,* 2014; O'Brien *et al.,* 2016; White *et al.,* 1993). As badgers may prefer to use field boundaries, and may also preferentially use particular fields, use of space close to water troughs was compared with use of space close to the boundaries of fields containing troughs (excluding the space surrounding troughs), rather than all space away from troughs. Taking into account the precision of badger GPS locations, 5 m buffers were createdaround both the water trough locations and the boundaries of the fields that contained water troughs, using QGIS software (QGIS.org, 2020), with areas ≤5 m from water troughs excluded from the field boundary buffer areas. Each badger GPS location was then classified as being located either ≤5 m from a trough, ≤5 m from the boundary of a field containing a trough (but >5 m from a trough), or outside both of these buffers. For each individual badger, we recorded the numbers of GPS locations falling into each of these categories.

To estimate the sizes of the field boundary and trough buffer areas for each individual badger, the home range of each badger was estimated using the *a-LoCoH* method (Getz *et al.*, 2007), following Woodroffe *et al.* (2016a) and Ham *et al.* (2019). Home range estimates were generated in *R* (R Core Team, 2014) using the package *tlocoh* (Lyons *et al.,* 2013), and using the 95% isopleth as the home range boundary. The area of water trough buffer and field boundary buffer within each individual badger's home range was then calculated using QGIS. Poisson regression was used to compare the numbers of GPS locations falling within the water trough and field boundary buffers. This analysis included, as fixed effects, the identity of each badger, and the (ln-transformed) area of each buffer within each badger home range, to account for variation in sampled area, but where the response was counts to a point rather than a visit rate per unit area. Poisson regressions were fitted in R (R Core Team, 2014).

Results

Camera trap monitoring

Camera trap monitoring was used to observe animal activity at 13 water troughs on five farms across three study sites. Although camera traps were deployed at 14 troughs on 5 farms, data could not be recovered from one camera, therefore recordings were collected from six *M. bovis* positive troughs and seven *M. bovis* negative troughs. The cameras generated 305 camera trap-days of data. During this period, 3,238 individual drinking events were recorded for sheep and 214 for cattle. Among wild species, 1,151 events were recorded for birds (only Corvidae, Columbiformes, barn owl (*Tyto alba)* and common buzzard (*Buteo buteo)* were recorded on more than one occasion), two events for badgers, and one for a red fox (*Vulpes vulpes)* and one for grey squirrel(*Sciurus carolinensis)* (Table 1.1). Badger, fox, and squirrel visits were not analysed due to low numbers of observations.

Poisson regression indicated that both sheep and wild birds made significantly more visits to *M. bovis* test-positive troughs than to test-negative troughs (Table 1.2). The model of cattle drinking events failed to converge due to insufficient data. These results were highly sensitive to inclusion of the farm id variable.

GPS tracking

GPS collars were fitted to 68 badgers on 20 farms, across all four study sites. GPS collar data were recorded between May 2013 and September 2017, generating 7,977 badger-nights of tracking data. During this period, there were 4,870 badger-nights with locations within the 5 m buffers of water troughs or 5 m buffers of field boundaries. There were 77,602 badger GPS locations recorded within the 5 m field boundary buffer, and 1,681 locations recorded within the 5 m water trough buffer.

There were significantly fewer locations recorded within 5 m of a water trough than within 5 m of a field boundary (Poisson regression; estimated effect of water trough versus field boundary = -3.91, standard error = 0.07, $P < 0.001$), after accounting for the effects of badger identity ($P < 0.001$) and buffer area (inm^2, natural log-transformed, estimate = -0.19, standard error = 0.06, $P < 0.005$).

Figure 1.1: Camera trap images showing examples of drinking events at monitored water troughs by domestic livestock and wildlife. A – Sheep *Ovis aries*, B – Magpie *Pica pica* (using the same water trough as A), C – Cattle *Bos taurus*, D - Grey squirrel *Sciurus carolinensis*, E - Barn owl *Tyto alba*, F - Common buzzard *Buteo buteo,* G - Badger *Meles meles*, H – Red fox *Vulpes vulpes*.

Table 1.1: Summary of the frequency of drinking events by livestock and wildlife at water troughs on five farms in Cornwall, U.K. Water trough *M. bovis* status was determined using the qPCR method described in King *et al.* (2015). Species identity incorporates multiple species of wild birds. In situ cameras were checked regularly but due to camera failure monitoring days were not always consecutive.

			Species					
Trough number (Farm)	***M. bovis* status of trough**	**Number of days monitored**	**Badger**	**Wild birds**	**Cattle**	**Fox**	**Sheep**	**Squirrel**
1 (C4-C)	Negative	25	0	324	0	0	1303	0
2 (C4-C)	Positive	4	0	6	0	0	283	0
3 (C4-C)	Negative	28	0	483	0	0	1218	0
4 (C4-C)	Negative	3	0	4	0	0	68	0
5 (C4-E)	Negative	40	0	29	67	0	0	0
6 (C4-F)	Negative	15	0	13	123	0	366	1
7 (F1-E)	Negative	9	0	0	0	0	0	0
8 (F2-A)	Positive	4	0	3	0	0	0	0
9 (F2-A)	Negative	47	0	62	0	0	0	0
10 (F2-A)	Positive	27	1	94	0	0	0	0
11 (F2-A)	Positive	37	1	34	0	0	0	0
12 (F2-A)	Positive	44	0	59	0	0	0	0
13 (F2-A)	Positive	22	0	40	24	1	0	0
Total		305	2	1151	214	1	3238	1

Table 1.2: Summary of Poisson regression models comparing variation in the number of drinking events for sheep and wild birds among water troughs with differing *M. bovis* test outcome. Numbers of camera-trap days of monitoring (ln-transformed), and farm identity, were included as fixed effects. The model of cattle drinking events failed to converge due to insufficient data.

Variable	**Sheep**				**Birds**			
	Estimate ± SE	X^2	DF	P	Estimate ± SE	X^2	DF	P
***M. bovis* test positive trough**	0.96 ± 0.12	-78.39	6,7	<0.001	0.29 ± 0.14	-4.68	6,7	0.03
Ln (days of monitoring)	1.29 ± 0.05	-1538	6,7	<0.001	1.77 ± 0.10	-795	6,7	<0.001
Farm	Farm B, 27.1 ± 3270 Farm C, 0.13 ± 9978 Farm D, 26.60 ± 3270 Farm E, 2.06 ± 9978	-94.89	6,10	<0.001	Farm B, 2.68 ± 0.14 Farm C, -0.66 ± 0.22 Farm D, 0.28 ± 0.33 Farm E, -16.68 ± 1276	-1237	6,10	<0.001

Discussion

Water troughs on cattle farms were seen to be used frequently, not just by livestock, but by multiple species of wild birds, though their use by badgers and other wild mammals was very rare. Our results show that only two instances of drinking events by badgers, one by a red fox and one by a grey squirrel, were recorded over 305 camera trap nights. GPS tracking of badgers confirmed their infrequent use of water troughs, with evidence that GPS-collared badgers avoided the space close to water trough locations, relative to other land close to the boundaries of the same fields. These results suggest that it is unlikely that badgers are using cattle water troughs regularly.

Cattle to cattle transmission of *M. bovis* plays an important role in the persistence of bTB in herds (Crispell *et al.,* 2019; Donnelly & Nouvellet, 2013; Green *et al.,* 2008). While opportunities for the direct transmission of *M. bovis* are high within groups of cattle (and so indirect transmission through the environment may be relatively less important), survival of *M. bovis* within water troughs may facilitate indirect transmission among cattle both within and between groups within a herd. Either sequential use of the same field (and hence trough) by different groups, or even use of adjoining field (when a single trough is placed so as to be accessible to two adjoining fields) would enable water troughs to act as fomites for indirect *M. bovis* transmission. Cattle testing protocols remove test-positive individuals from herds at the point of test, however, *M. bovis* excreted from infected individuals might remain in trough water and pose an ongoing risk to cattle, for weeks or months after the test- positive individual has been removed from the farm environment (Williams and Hoy, 1930; Young *et al.,* 2005; Fine *et al.*, 2011). The possibility of a false negative bTB test results (Monaghan *et al.*, 1994) also creates more opportunities for *M. bovis* to be shed into the environment and may increaserisk of cattle to cattle transmission.

Failure to detect regular badger use of water troughs, using either GPS collars or camera traps, suggests that in our study area, water troughs were not a key resource for badgers. Although both methods of observation gave complementary results, our GPS tracking data would suggest a greater visitation rate of badgers to water troughs than would our camera trapping results. GPS-collars were fitted to only a subset of adult badgers at each farm and so the number of badger visits to troughs is estimated for only a sample of the available badgers. However, GPS data do not enable us to determine if badger presence close to a trough involved a drinking event. In contrast, camera trapping enables capture of all badger visits to water troughs anddetermination of whether or not drinking occurred.

Badgers may not be reliant on water troughs as a water source in this area, instead relying on natural sources (food, surface water) instead. Previous studies in both the Republic of Ireland and Northern Ireland likewise found that badgers did not regularly use water troughs (O'Mahony, 2014; Campbell *et al.*, 2019). Campbell *et al.* (2019) found that badgers utilized cattle water troughs in fields once every 87 days compared to this study where a rate of one visit per trough every 153 days was observed. Badger use of other farm resources has been shown to vary considerably among farms (Tolhurst *et al.*, 2009; Ward, *et al.*, 2010; Judge *et al.*, 2011; Woodroffe *et al.*, 2017; Robertson *et al.*, 2019), most likely based on badger population density and resource availability. However, unlike food, water is unlikely to be a resource that is limiting for badgers, even in high density populations, and so we might expect the GPS- tracking results to be representative of wider areas. However, camera trapping water troughs might be expected to detect greater rates of badger visits inhigher density, or drier, areas.

Despite only two badger drinking events being recorded at two water troughs during the course of this study, badger use of troughs could still contribute to interspecific transmission of *M. bovis* because the bacteria may persist in water for up to 58 days (Fine *et al.*, 2011). Other species were observed using water troughs, which may pose a risk of *M. bovis* exposure to both wildlife and cattle. Although our statistical analyses showed significantly more sheep and wild birds drinking at test-positive water troughs, this result should be treated with caution due to the limited number of farms in the study. Exploratory analyses revealed that model outcomes were highly sensitive to the inclusion of farm identity as a fixed effect, suggesting that more robust conclusions could be reached by repeating the study using troughs from a larger number of farms. Although *M.* bovis has been recorded in sheep (Malone *et al.,* 2003; Mendoza *et al.,* 2012), Case control studies have not found associations between keeping sheep and increased bTB risk for cattle (Broughan *et al.*, 2016).

Guidelines on bTB biosecurity best practice state that both cattle feed and water troughs should have a top lip height of >90cm (TB Hub, 2017). Studies have observed badgers climbing up to use cattle feed troughs of up to 115cm (Garnett *et al.,* 2002) and/or standing on their hind legs to access troughcontents (O'Mahony, 2014). Young cattle or mixed species grazing (e.g., cattle and sheep) may also need lower heights to enable use of troughs. Although it is also recommended that troughs are placed without adjacent structures to enable climbing (TB Hub, 2017), this is often impractical; troughs are usually placed in field boundaries as plumbing pipes often run along hedges and one trough regularly spans both sides of the hedge so it can be used by two fields. We observed a badger drinking from a water trough using the field boundary (hedgerow) to gain elevation and therefore access, and this was also observed by Campbell *et al.* (2019). This study showed no regular use of water troughs, so raising water troughs to the recommended height and moving them away from adjacent structures such as field boundaries is unlikely to substantially affect the risk of *M. bovis* transmission between badgers and cattle.

To conclude, although unlikely to play a pivotal role in badger to cattle transmission of *M. bovis*, water troughs are still regularly used by livestock and wild birds and therefore constitute a potential location for the indirect transmission of infections, via fomites. Regular cleaning, disinfection and avoidance of stagnation of water troughs should be advised, especially if test- positive cattle are detected in the herd (Allen *et al.,* 2021). As the risk of infection is currently unknown, future work conducted into the survival of *M. bovis* in water troughs will help towardsunderstanding the role that water troughs may play in maintenance of infection on farms and in cattle to cattle transmission.

Chapter 3:

Spreading of farm manure and slurry in relation to bovine tuberculosis.

Abstract

Mycobacterium bovis, the causative agent of bovine tuberculosis (bTB), is shed in the faeces of infected cattle and has been shown to persist in the environment. Uncertainty regarding the means of indirect transmission between hosts in their shared environments hampers bTB management strategies. Farmers often dispose of faecal waste from housed cattle by spreading it on fields as manure or slurry. When a cattle herd has a positive bTB test result, thefarm is placed under restrictions that limit the movement and trade of theircattle, but there are no statutory restrictions on the spreading of faecal waste. We used GPS trackers to monitor cattle manure and slurry spreading vehicles on farms with and without bTB restrictions. Ten vehicles monitored for 425 vehicle-days of manure- or slurry-spreading engaged in 171 (40%) vehicle-daysof spreading on land neither owned nor rented by the farmer, with a mean distance of 5072 m (range – 219 m – 22886 m) from the home location. This tendency to spread manure and slurry on other properties, distant from the home location, did not change in response to the imposition or lifting of bTB restrictions. Our study highlights the potential contribution of human-mediated dispersal of cattle faecal waste to transmission of *M. bovis*.

Introduction

Bovine tuberculosis (bTB), caused by infection with *Mycobacterium bovis*, is an endemic and costly disease of livestock in the UK (DEFRA, 2014; TBSPG, 2016; Welsh Government, 2017). Taxpayers in the England spend an estimated £120 million per year tackling the disease, with costly testing and compensation schemes (Godfray *et al*., 2018). Farmers bear additional financialburdens after a bTB positive test result due to government control measures, including lost earnings from being unable to sell movement restricted cattle, andadditional costs of feed and housing required to keep unsellable livestock (DEFRA, 2014).

As well as causing disease in cattle, *M. bovis* can infect many domestic and wildlife species, and is a zoonotic infection, making it a public health concern (Biet *et al*., 2005; Grange & Yates, 1994; Neill *et al*., 1994). Tuberculosiscaused by *M. bovis* in humans was widespread in the UK until the 1950s when pasteurisation was introduced, leading to a dramatic reduction in the incidence of human cases (De La Rua-Domenech, 2006). In the UK, *M. bovis* infectionhas been found in a number of wildlife species (Delahay *et al.,* 2001), badgers (*Meles meles*) have been proven to play a role in wildlife to cattle transmissionof *M. bovis* (Donnelly and Nouvellet, 2013). The presence of infection in multiple species of deer may also pose considerable localised risks (Crispell *et al*., 2020; Ward *et al*., 2009). Uncertainty surrounding the mechanisms of interspecific transmission of *M. bovis* (Woodroffe *et al*., 2016) mean that management strategies to minimise the risk of transmission are not well targeted.

M. bovis bacteria can be shed by the host in urine, faeces and respiratory tract secretions (Neill *et al.*, 1994). *M. bovis* bacilli are able to persist in the environment and are variously able to withstand dehydration, change in temperature, change in pH and the effects of sunlight (Duffield and Young, 1984). *M. bovis* has been cultured after four months in the environment and *M. bovis* DNA has been detected up to 21 months after initial contamination,indicating the presence of potentially viable bacilli (Williams & Hoy, 1930;Young *et al.*, 2005). The presence of *M. bovis,* and its persistence in theenvironment provides the opportunity for disease transmission to both cattleand wildlife (Courtenay *et al*., 2006; Fine *et al*., 2011).

Dung arising from cattle farming is both an important waste product and a sustainable resource; when applied to fields, cattle manure (solid cattle waste) and slurry (liquid cattle waste containing faeces and urine) can contribute to good soil quality and help to address crop nutrient requirements (Chambers *et al*., 2000). However, spreading manures may constitute a potential hazard for bTB transmission when it comes from farms that have, or have recently had, infected cattle (Green & Cornell, 2005; Phillips & Foster, 2000). *M. bovis* has been detected in the faeces of infected cattle (Williams and Hoy, 1927; Neill *et al.*, 1994; Jha *et al.*, 2007). *M. bovis* has also been shown to survive in experimentally stored liquid cattle waste for up to six months (Scanlon and Quinn, 2000) and can be aerosolised through spreading, and detected up to 50 m from the source (Hahesy *et al*., 1992).

Cattle manures are generally spread onto the surfaces of fields using manure spreaders and slurry tankers. These are pieces of heavy machinery towed behind a tractor, which operate in different ways. Slurry tankers contain slurry which has been stored in a slurry pit prior to being pumped into the tanker. Slurry is typically delivered onto fields through a directional spraying mechanism. Manure spreaders contain more solid cattle waste which has been stored in a pile, usually situated either in a cattle yard or field. The manure is spread using an open sided tank with rotating chains that expel the waste.

Current bTB controls involve routine skin testing of cattle herds (DEFRA, 2014; Welsh Government, 2017). Herds are considered 'Officially TB free' when they have two consecutive tests with no test positive cattle, this enables farmers to transport and sell cattle. If any individual within a herd has a positive test result, the whole herd is placed under movement restrictions, which prevents the movement of cattle from the herd other than movements to slaughter or to other herds in some specific circumstances subject to licence (AHVLA, 2014; DEFRA, 2014). Although legal constraints are placed on the movement of cattle when they are placed under restriction, no such legal constraints apply to the handlingof cattle manure or slurry. The information given to farmers when they are informed of a positive test states: "*whilst your farm is under TB movement restrictions, manure and slurry must not be removed to other farms without priorwritten authorisation from APHA*". However, this advice is not formally part ofthe restriction notice and is therefore not legally enforceable (APHA, pers.comm., 2019). A recent independent review of bTB control strategy concluded that '*the potential for bovine TB to be dispersed by spreading slurry or manure on the land may have been under-appreciated*' (Godfray *et al.*, 2018).

During this study we used GPS technology to track the movements of manure spreaders and slurry tankers in an area with high incidence of bTB in cattle farms.

Specifically, we aimed to characterise practices of spreading cattle manures on farmland and to assess whether such practices changed when farms were placed under bTB restrictions.

Methods

All data were collected from 10 farmsdistributed among four study sites (F1 – three farms, F2 – one farm, C2 – fourfarms, C4 – two farms) in Cornwall, South West England, between 2014 and 2018, for full descriptions of study sites see Woodroffe et al., 2016a. Sites C2 and C4 were located inland in north and east Cornwall in areas of mixed livestock (cattle and sheep) grazing and arable land. F1 was a coastal site situated in west Cornwall consisting of cattle pasture and grazed scrubland. F2 was a coastal site in south Cornwall predominantly containing cattle grazing alongside arable areas. All monitoring was carried out with the farmers' permission. All farm boundaries were mapped by the farmers.

To monitor movement, GPS-tracker units (iGotU GT600, Mobile Action Technology Inc., Taipei, Taiwan) with rechargeable batteries were attached to the manure spreader and/or slurry tanker at each farm. Each tracker was enclosed in a sealed plastic bag and securely attached using strong adhesive tape. The dates of attachment and removal were recorded in all cases, and these dates defined each 'deployment period'. Trackers were replaced regularly to ensure consistent monitoring within the constraints of limited battery life. For data analysis, one tracker-day was defined as a 24-hour period (0000h-2359 h) for each tracked vehicle. Trackers were triggered to start recording data by an internal motion sensor as soon as the vehicle started to move. The tracker then recorded locations at pre-programmed intervals (varying between 5 s and 120 s depending on the deployment) until the vehicle stops and the motion sensor stops recording. The tracker motion sensors were highly sensitive, and at least one location was recorded on 1,636 (58%) of the 2,820 days when trackerswere deployed, even though multiple consecutive locations, indicating movement, were recorded on only 513 days (18%). To exclude days within the deployment periods when trackers were potentially not recording data due to battery failure, trackers were assumed to have been able to record manure- or slurry-spreading activity from the time of deployment, on all days up to and including the day when the last location was recorded; this was defined as the ‘monitoring period’.

Using GIS software (QGIS.org 2018. Open-Source Geospatial Foundation Project. https://qgis.org), each vehicle was assigned a “home” location, defined as the space where the machinery was stored when not in use, typically in a farmyard or shed. These home locations were observed to be the same for each vehicle throughout each monitoring period. For each spreading day, the furthest straight-line distance from this home location was measured. Spreading days were identified as those when a minimum of 10 consecutive GPS- locations were recorded within the same field. These spreading day locations displayed two distinct patterns; when GPS locations were taken with 5 s intervals, the back-and-forth movement path characteristic of spreading was easily visible (Figure 2.1, A). When GPS locations were taken with 120 sintervals a minimum of 10 consecutive locations in a field (i.e., ≥20 mins spent in the field) was used to indicate spreading (Figure 2.1, B). Spreading days were further classified according to whether spreading occurred within the farmers property boundaries (termed “own land”, and including any land held by the same farmer, whether or not it was remote from the main holding), or on land confirmed to be neither owned nor rented by the farmer (termed “elsewhere”). Where spreading was recorded “elsewhere”, farmers were contacted to confirm that their boundaries had been recorded correctly.

For each day of monitoring, we recorded the farm’s bTB status (under restriction due to bTB test-positive cattle, or not under restrictions) using the dates that bTB restrictions were imposed and lifted, as recorded on the Animal and Plant Health Agency’s bTB information website (https://www.ibtb.co.uk).

To test the hypothesis that farmers altered their slurry- or manure-spreading practices when under restriction, we first constructed a base model for each of three outcome variables: (i) whether or not spreading occurred on a particular day during the monitoring period; (ii) whether such spreading occurred within the farmer’s own farm boundaries or ‘elsewhere’; and (iii) the maximum straight-line distance from the home location on each spreading day. We considered three candidate explanatory variables: farm type (beef, dairy or mixed), vehicle type (manure spreader or slurry tanker) and time of year (Jan-Feb, Mar-Apr, May-Jun, Jul-Aug, Sep-Oct or Nov-Dec). All models included farm identity as a random effect, to account for repeated journeys by vehicle(s) from the same farm, all farms were treated as independent samples even when they were in the same study area. Step down simplification of each model was conducted, retaining only statistically significant variables in each model. Once the base model for each outcome variable was constructed, we then added a term describing the farm's bTB status at the time of observation (under restriction or not under restriction). All models were fitted using generalised linear mixed effects models (GLMM) in R using the package lme4 (Bates *et al.*, 2014; R Core Team, 2014). For binary outcome variables (whether spreading occurred, and whether such spreading occurred on farms' own land or elsewhere) we used models with binomial error distribution, and for the continuous outcome variable (maximum distance from home) we used a model with Gaussian error distribution.

Results

Trackers were deployed for a total of 2,820 days across 13 vehicles on 10 farms (Table 2.1). The total monitoring period (excluding days with no data due to potential battery failure) was 2,312 tracker-days, representing 82% of the deployment period. Spreading was detected on 425 (18%) tracker-days.

Spreading activity was not confined to the study farms themselves. 171 (40%) spreading days involved spreading on land that was confirmed to be neither owned, nor rented by the farmers responsible for the vehicles. There was substantial variation in the distance from the home location where spreading occurred. On spreading days, the trackers travelled an average straight-line distance of 1,282 m (range 120 m – 8,962 m) from their home location when restricted to their own farm, and 5,072 m (range 219 m – 22,886 m) on days when they visited land that was neither owned nor rented by the farmers. Maps show some trackers recording spreading large distances from their home location (Figure 2.1 C) whilst others remained within their home boundaries throughout the monitoring period (Figure 2.1 E).

All three base models included the time period variable, the base model for spreading elsewhere also included vehicle type (Table 2.2). Farm type (beef vs dairy) was not included in any of the three base models. After adjusting for these base model variables, there was no effect of farm bTB status on any of the three outcome variables (Table 2.2). Maps confirmed that patterns of movement, including long-distance movement away from the home farm, were similar irrespective of whether or not farms were under bTB restriction (e.g., Figure 2.1 D). Spreading on the home farm was conducted on both pasture and arable fields. Information on land use (arable or pasture fields) of spreading locations away from the home farm was unavailable.

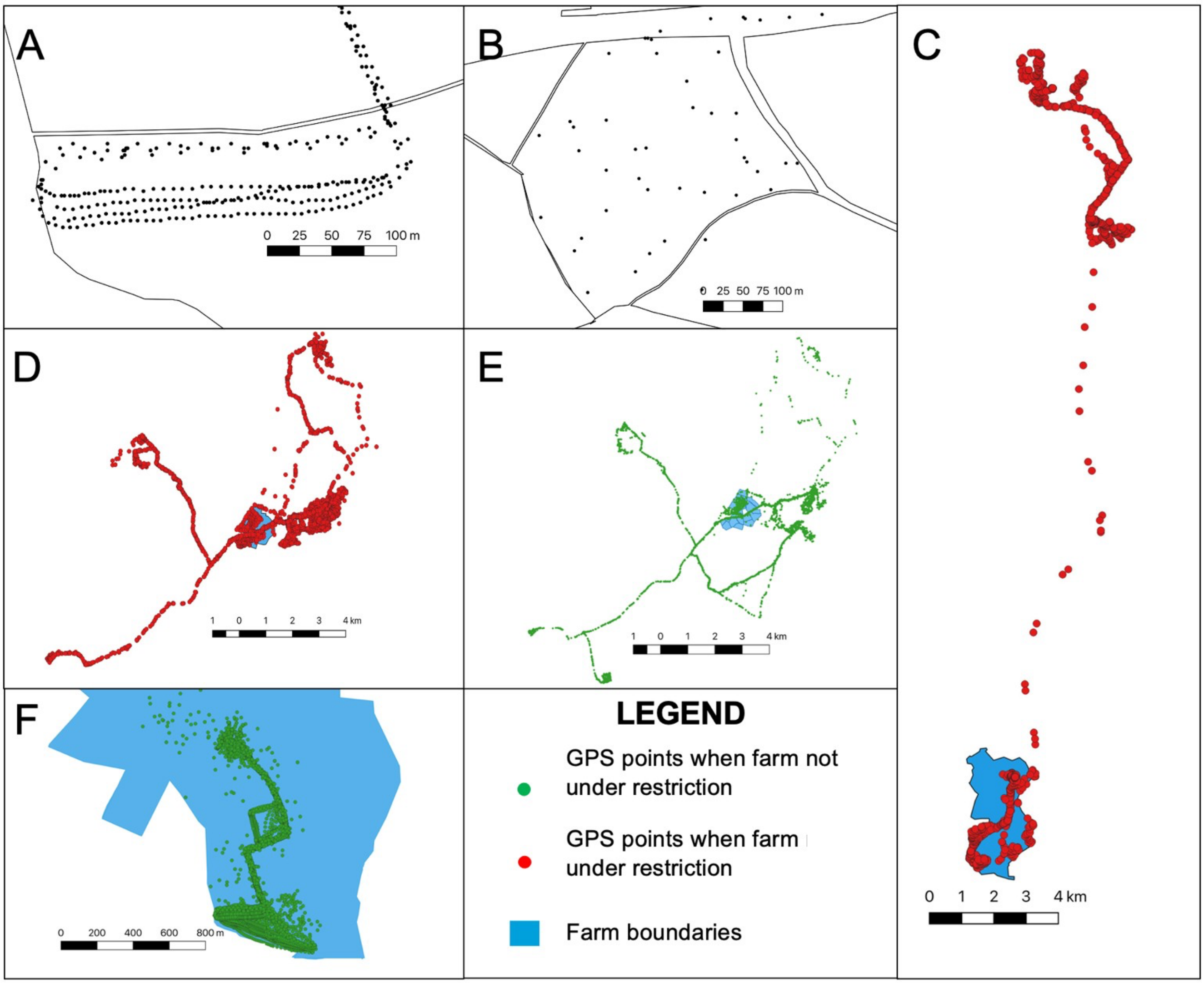

Figure 2.1. Examples of GPS tracking of manure spreaders and slurry tankers in Cornwall, UK. Dots represent individual GPS fixes. Home farms are shown in blue. A and B) Typical movement patterns of manure spreader and slurry tankers while actively spreading. Spreading events were identified by recording ≥10 consecutive locations within a single field, with the exact pattern varying according to whether GPS points were recorded at high frequency (Panel A, 5 second interval) or lower frequency(Panel B, 120 second interval). C) Long distance movement from home location. D and E) Movement patterns on the same farm, when subject to bTB restrictions (Panel D – red dots) and when Officially TB Free (Panel E – green dots). F) Vehicle remaining within its own farm boundaries.

Table 2.1: Study farm information and number of days of tracking manure spreaders and slurry tankers.

				Days of monitoring		
Farm ID	**Farm Area km^2**	**Farm type**	**Vehicle type**	*No bTB Restriction*	*Under bTB Restriction*	*Total*
C2A	0.95	Beef	Manure spreader	4	45	49
C2B	0.76	Beef	Manure spreader	0	124	124
C2C	0.88	Dairy	Manure spreader	112	0	112
C2D	1.75	Beef & Dairy	Manure spreader	29	110	139
			Slurry tanker	0	104	104
C4A	0.51	Dairy	Slurry tanker	177	283	460
C4B	0.53	Dairy	Manure spreader	229	259	488
			Slurry tanker	28	124	152
F1A	1.12	Beef	Manure spreader	1	44	45
F1B	1.71	Dairy	Manure spreader	65	0	65
			Slurry tanker	22	0	22
F1C	1.28	Beef	Manure spreader	237	139	376
F2A	2.37	Dairy	Manure spreader	176	0	176
			Total	904	1408	2312

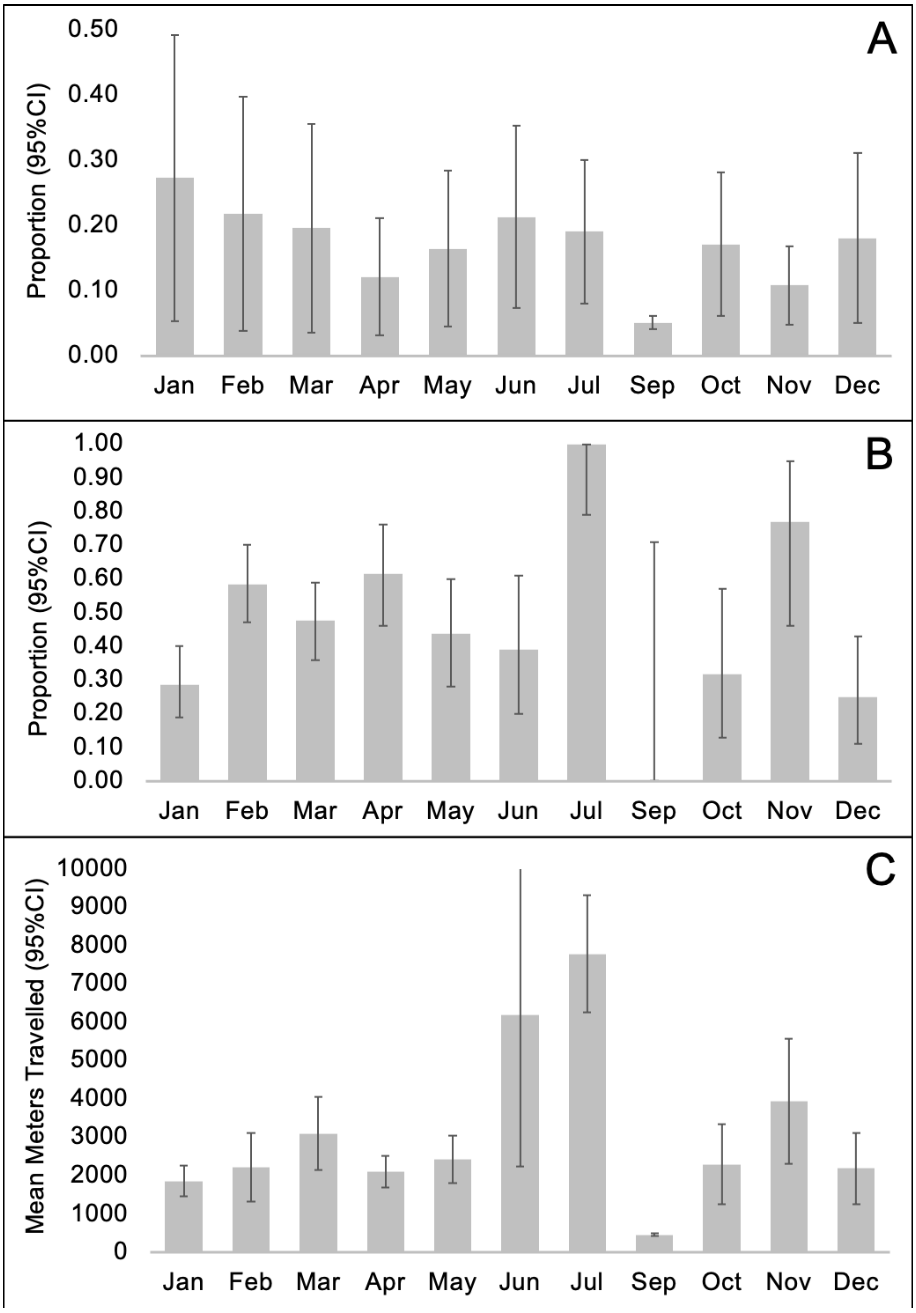

Figure 2.2. Seasonal variation in the movements of slurry tankers and manure spreaders from 10 cattle farms in Cornwall. A – Proportion of spreading days per month with exact binomial 95% confidence intervals. C – Proportion of spreading days that occurred away from home farm per month with exactbinomial 95% confidence interval error bars. D – Mean distance (m) travelled from the home location each month with 95% confidence interval error bars. Panels exclude the month of August when no data were recorded.

Table 2.2: Effects of bTB restriction on three measures of manure and slurry spreading practices. All three models are generalised linear mixed effects models including farm identity as a random effect, with binomial error distribution for the “spreading vs not spreading” and “spreading elsewhere vs own land” outcome variables and Gaussian error distribution for the “maximum distance from home” outcome variable. Effects sizes for base model variables are shown for the base model only, excluding the bTB status variable.

Variable		**Spreading vs not spreading**				**Spreading elsewhere vs own land**				**Maximum distance from home (m)**			
		Odds Ratio (95% CI)	X^2	DF	P	Odds Ratio (95% CI)	X^2	DF	P	Estimate □(95% CI)	X^2	DF	P
Vehicle type (slurry tanker vs manure spreader)		–	–	–	–	0.24 (0.070 – 0.81)	6.65	1	0.01	–	–	–	–
Time Period (relative to Jan/Feb)	Mar/Apr	0.72 (0.53 - 0.96)	15.88	5	0.01	1.35 (0.71 – 2.57)	14.02	5	0.02	627 (-151– 1404)	18.90	5	0.002
	May/Jun	1.03 (0.71 - 1.50)				0.88 (0.40 - 1.93)				157 (- 839 – 1153)			
	Jul/Aug	1.37 (0.71 - 2.63)				5.03 (1.36 – 18.68)				3436 (1628 – 5245)			
	Sep/Oct	0.64 (0.39 - 1.07)				0.83 (0.20 – 3.40)				-15 (-1448 – 1418)			
	Nov/Dec	0.60 (0.40 - 0.90)				3.49 (1.32 – 9.18)				580 (- 481 – 1640)			
bTB status (under restriction vs no restriction)		0.89 (0.65 - 1.21)	0.57	1	0.45	1.17 (0.55 – 2.46)	0.17	1	0.68	-260 (-1149 – 628)	0.30	1	0.59

Discussion

Our observations indicated that bTB restrictions had no detectable effect on the practices of spreading cattle manure or slurry. Some vehicles spread cattle manure or slurry large distances from the 'home' farm, including on land not managed by the same farm enterprise. This behaviour appeared not to change in response to bTB restrictions.

The maintenance of continued spreading practices has implications for the distribution of *M. bovis* in the environment. It has been shown that *M. bovis* can be shed by cattle into faeces (Williams and Hoy, 1927; Neill *et al.*, 1994; Jha *et al.*, 2007), and *M. bovis* bacilli have been detected in both manure and slurry (Scanlon and Quinn, 2000). Additionally, *M. bovis* has been shown to survive in the environment for extended periods of time (Courtenay *et al.*, 2006; Fine *et al.*, 2011; Williams & Hoy, 1930; Young *et al.*, 2005). Pasture contamination by the spreading of cattle slurry and manure could therefore provide an opportunity for indirect transmission of *M. bovis* both within and between farms.

Direct grazing of slurry/manure treated pasture present the greatest risk of *M. bovis* infection to grazing cattle. (Christiansen *et al.,* 1993). The current adviceis that cattle slurry/manure should be stored for six months prior to spreading and pasture should not be grazed by cattle for a minimum of two months (TB Hub, 2017). In a recent farming practices survey 45% of participating farmersdo not always or never store slurry for six months before spreading and only 30% of farmers thought that keeping grazing land free from slurry would reduce bTB risk (DEFRA, 2019). Cattle feeding in pasture fields recently treated with slurry are at risk of ingesting or inhaling *M. bovis* present either on vegetation orin the top layer of soil (Healy, 1968).

The production of silage from contaminated fields constitutes another possible *M. bovis* infection source (Allen *et al.,* 2021). Although the ensiling process creates an anaerobic environment hostile to *M. bovis* survival (Elferink *et al.,* 2000), bacilli are able to withstand dehydration, change in temperature and changes in pH (Duffield and Young, 1984; Rodríguez-Hernández *et al.*, 2016) and there is some evidence that use of silage clamps increases *M. bovis* risk (O'Hagan *et al.,* 2016; Skuce *et al.,* 2012). There is limited *M. bovis* survivaldata for silage however, US research indicated *M. bovis* remained viable on ensiled forages for white-tailed deer *Odocoileus virginianus* for at least 16 weeks (Palmer and Whipple, 2006; Grooms *et al.*, 2019).

The practice of slurry and manure spreading on arable fields may still pose a risk to cattle due to the aerosolisation of *M. bovis* bacilli. Contaminated aerosol created through the physical mechanisms of spreading have been detected up to 50m from the source (Hahesy *et al.*, 1992). Whilst these droplets of potential infection are likely to pose less of a risk to cattle than spreading directly on pasture, it presents a potential mechanism for the transmission of *M. bovis* both within and between neighbouring farms.

Practices such as slurry and manure spreading have the potential to distribute pathogens beyond the movements of cattle themselves (McCallan *et al.*, 2014). Manure spreaders and slurry tankers can be shared with other farms or contractors with hired equipment employed. Only one incidence of this was recorded during monitoring, where a farmer loaned a slurry tanker to another farm. A study in Northwest England showed that contractor vehicles visited more than half of the farms in the study area, yet cleaned and disinfected their vehicles only infrequently (Brennan & Christley, 2012). Continuing to spread potentially infected cattle waste on other properties and over large distances during herd restrictions could allow the dissemination of *M. bovis* into the wider environment away from the confines of individual farms. Our study suggests that these distances (up to 23km) included time spent on non-home farms. Spreading cattle slurry and manure on land that is neither owned or rented during herd restriction requires special dispensation from APHA, although it is counter to current government advice (DEFRA, 2019), and no examples of special dispensation were recorded in the present study.

Our findings that cattle manure and slurry have the potential to be transported over large distances may contribute a novel hypothesis for the dynamics of the geographical distribution and spread of *M. bovis* genotypes. Distances travelled by manure spreaders and slurry tankers (averaging 5072 m (range – 219 m – 22,886 m)) are greater than those travelled by badgers from a single social group (528 m (95% CI: 522-534m)(Ham, 2020)), but less than the distance individual cattle are likely to be transported when sold (Gilbert *et al*., 2005;Smith *et al*., 2003), and could therefore aid in the spread of *M. bovis* over intermediate distances. Previously the regional pattern of *M. bovis* strain distribution has been attributed to the movements of badgers, over a short distance, and cattle, over a short or very large distances (Smith *et al*., 2003). However, it is possible that the movement of cattle manure and slurry over intermediate distances might also be considered as a potential factor in explaining the distribution of *M. bovis* strain types and, hence, *M. bovis* transmission.

Spreading potentially infected slurry and manure into the environment might also be associated with introduction of infection to resident wildlife, which could later lead to transmission back to cattle (Phillips *et al*., 2003). Seeding ofinfection from wildlife into livestock populations is important in disease dynamicsand hampers disease eradication efforts, for example the onwards transmission between cattle caused by an initial badger-to-cattle transmission event is estimated to be responsible for 38% of cattle herd incidence (Donnelly & Nouvellet, 2013).

Although slurry and manure present a potential infection risk (Green & Cornell, 2005; Phillips & Foster, 2000), they also provide an important resource. When used as a fertiliser, slurry or manure can increase yield and provides a natural, cheaper and a potentially organic, alternative to chemical fertilisers (Chambers *et al*., 2000). Opportunities to reduce the risk posed by slurry/manure are available. Cattle slurry/manure should be stored for six months prior to spreading to reduce the risk if infectiousness (TB Hub, 2017). During a surveyof slurry/manure practices farms reported limited storage capacity; 16% of dairy farms and 25% of beef farms had storage capacity of less than one-month (Smith *et al*., 2001), our study farms also had limited storage capacity, with all monitored farms only having one slurry pit. The difficulties of storing cattle manures for sufficient timeto reduce the risk of *M. bovis* contamination could increase risk. Chemical disinfection of slurry using lime (calcium oxide/hydroxide) can be used (Scanlonand Quinn, 2000) and shallow injection practices can be used during slurry spreading to reduce the risk of aerosolisation (Allen *et al.,* 2021).

Despite bTB controls being in place in the UK for many years the disease is far from being eliminated, the number and geographical extent of cattle herds infected with *M. bovis* had increased since the 1970s and has only recently shown signs of decline (DEFRA, 2020a). Our study highlights the potential for human-mediated dispersal of cattle waste to play a role in the spread of *M.bovis* over intermediate distances. Firstly, we show a need for clearer more emphasized information for farmers, allowing them to understand the risks of spreading cattle waste. Secondly, whilst we acknowledge that guidelines on storage and spreading of cattle waste exist, we suggest that these could be reviewed and strengthened to help prevent future cattle herd infections.

Chapter 4: General Discussion

Indirect transmission of *M. bovis* is known to be important in interspecific transmission between badgers and cattle (Drewe *et al.*, 2013; Woodroffe *et al.*, 2016), and may also play a role in the maintenance of infection within cattle herds. Within this thesis, I have investigated two potential transmission routes of *M. bovis* in the farm environment, that might pose an infection risk to both wildlife and cattle: water troughs and cattle manure and slurry.

First, I found that cattle water troughs were utilised by livestock and a variety of wild bird species, though their use by wild mammals was rare. This is in keeping with previous studies conducted elsewhere in the UK and Ireland which also recorded low visitation rates of badgers to cattle water troughs (Garnett *et al.,* 2003; O'Mahony, 2014; Campbell *et al.*, 2019). Whilst these results suggest that interspecific transmission of *M. bovis* via water troughs might be unlikely, the potential for *M. bovis* to remain infectious for up to 52 days within water (Fine *et al.*, 2011) raises the possibility of troughs being an important location for cattle to cattle transmission. This cattle to cattle transmission could possibly still occur for some time after a test-positive individual is removed from the herd following a bTB test, as bacteria excreted by the individual, before removal, may remain within the farm environment for some time. The possibility of a false negative bTB test results (Monaghan *et al.*, 1994) also creates more opportunities for *M. bovis* to be shed into the environment and may present an increased risk to cattle.

Second, I found the practices of spreading cattle manure and slurry, which occurred both on the home farm and on land not owned or rented by the home farmer, did not vary in response to the farm recording a positive bTB test result. The application of potentially infectious cattle excreta into the environmentcreates an opportunity for indirect transmission from cattle to both cattle and wildlife, both at the home farm and elsewhere, on land visited by manure and slurry spreaders.

Despite management plans being in place since the 1950s (De La Rua-Domenech, 2006), bTB continues to be among the most important livestock diseases in the UK (DEFRA, 2020a). However, routes of transmission are still not fully understood and the role that environmental sources of *M. bovis* play in both interspecific and intraspecific transmission are still being uncovered. Initially it was assumed that badger to cattle transmission occurred via direct contact between individuals, however, with the use of new technologies including GPS collars and proximity sensors it was discovered that such direct meetings are rare (Drewe *et al.*, 2013; Woodroffe *et al.*, 2016). As a result, it is now widely accepted that interspecific transmission is most likely to occurindirectly, via a contaminated shared environment.

Transmission of *M. bovis* between cattle is less studied than transmission between badgers and cattle. Amongst individuals within the same group (for example milking cows or bullocks) opportunities for direct contact are plentiful; however, contact between different groups of cattle on a farm, between current and previous cattle in a group or between herds on different farms is unlikely to involve direct contact. Environmental sources of *M. bovis* such as within water troughs or in faeces spread onto pasture fields, may provide a mechanism through which cattle can become infected as a result of indirect contact with other cattle. This has potential implications on within and between farm disease dynamics, especially if it has been previously assumed that any *M. bovis* excreted by individual cattle is removed at the same time as the individual, instead of the possibility of extended survival.

Both water troughs and slurry and manure spreading are covered in advisory guidance issued by Government for prevention of disease transmission. It is recommended that water troughs use mains water and are regularly cleaned to prevent infection with *M. bovis* (TB Hub, 2020). However, no additional advice regarding water troughs is given to farmers following a positive bTB test result. It is also advised that slurry and manure are stored for at least six months before spreading, pasture land is not grazed for two months after spreading (TB Hub, 2017), and that waste from test-positive cattle is only spread on the home farm (DEFRA, 2019). However, these recommendations are not enforceable and are adhered to at farmer discretion. In light of the potential for water, slurry and manure to contribute to the maintenance of *M. bovis* infection it would be prudent for farmers and policymakers to consider the potential risks and the guidance given by Government organisations on these aspects of biosecurity. Additionally, it might be necessary for the guidance to be strengthened and/or become enforceable so that every effort is made to reduce opportunities for disease maintenance and transmission.

As found in bTB disease management, failure to understand transmission routes of pathogens into domestic livestock species can hamper disease controlin other epidemiological systems. The cause of *Brucella abortus* infection in cattle surrounding Yellowstone National Park has been widely debated, with infection from wild bison (*Bison bison)* thought to be the most likely route.

However, recent genomics has revealed that elk (*Cervus canadensis)* to cattle transmission is more likely (Kamath *et al.*, 2016). To further narrow downpotential locations of elk to cattle contact, GPS collars have been fitted to elk and combined with data on cattle grazing locations and trough locations to determine when and where direct or indirect pathogen transmission might occur(Pruvot *et al.*, 2020). Management programmes in this system should reflect this and focus on reducing opportunities for contact between cattle and elk.

The rapid identification of transmission pathways and translation of this understanding into management guidance is particularly important when new disease emerge, to which a population has no immunity. The initial advice to prevent the spread of COVID-19 in the UK revolved around handwashing as well as maintaining social distancing. It was later found that the virus could become airborne (Morawska and Milton, 2020) and transmitted through inhalation and therefore that handwashing was insufficient to control the spread of the pathogen (Ma *et al.*, 2020). Further research concluded that wearing face masks was beneficial in reducing transmission (Burnett and Sergi, 2020), however, the translation of this into policy to protect the general public from infection was slow, resulting in increased numbers of infections.

Where transmission routes are identified and control measures designed, communities might be reluctant to adhere to the guidelines due to their conflict with cultural norms. Ebola virus, a zoonotic disease affecting humans, is excreted in bodily fluids and human corpses are highly infectious (Judson *et al.,* 2015). Therefore a key method of reducing transmission opportunities is toavoid contact with potentially infectious people whilst sick or deceased. However, funeral traditions in west Africa entail extended contact with thedeceased and the items used to care for them whilst they were sick. Successful prevention of transmission via this route requires careful, discrete education of communities about virus transmission and the adaptation of guidelines to be accepted by affected communities (Whitty *et al.*, 2014; Adongo *et al.*, 2016).The need for trust and understanding between affected communities and those trying to prevent infection transmission and impose guidelines is vital for the successful management of all diseases.

Zoonotic diseases such as bTB, COVID-19 and Ebola have serious health, welfare and economic implications around the world. Successful management of such diseases relies on a number of steps; understanding where, when and how transmission events are likely to occur, designing effective mitigation techniques to prevent transmission opportunities and education and guidance to affected communities to ensure that management procedures are adhered to. Where there is failure in any of these steps, rapid reductions in transmission events and hence effective control of disease are unlikely to occur.

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