



REVIEW ARTICLE

Mycobacterium bovis: Characteristics of Wildlife Reservoir Hosts

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Summary

Mycobacterium bovis is the cause of tuberculosis in animals and sometimes humans. Many developed nations have long-standing programmes to eradicate tuberculosis in livestock, principally cattle. As disease prevalence in cattle decreases these efforts are sometimes impeded by passage of *M. bovis* from wildlife to cattle. In epidemiological terms, disease can persist in some wildlife species, creating disease reservoirs, if the basic reproduction rate (R_0) and critical community size (CCS) thresholds are achieved. Recognized wildlife reservoir hosts of *M. bovis* include the brushtail possum (*Trichosurus vulpecula*) in New Zealand, European badger (*Meles meles*) in Great Britain and Ireland, African buffalo (*Synacerus caffer*) in South Africa, wild boar (*Sus scrofa*) in the Iberian Peninsula and white-tailed deer (*Odocoileus virginianus*) in Michigan, USA. The epidemiological concepts of R_0 and CCS are related to more tangible disease/pathogen characteristics such as prevalence, pathogen-induced pathology, host behaviour and ecology. An understanding of both epidemiological and disease/pathogen characteristics is necessary to identify wildlife reservoirs of *M. bovis*. In some cases, there is a single wildlife reservoir host involved in transmission of *M. bovis* to cattle. Complexity increases, however, in multihost systems where multiple potential reservoir hosts exist. Bovine tuberculosis eradication efforts require elimination of *M. bovis* transmission between wildlife reservoirs and cattle. For successful eradication identification of true wildlife reservoirs is critical, as disease control efforts are most effective when directed towards true reservoirs.

Introduction

Zoonotic diseases are responsible for most (60.3%) emergent diseases of humans. Moreover, the preponderance (71%) of emerging pathogens is of wildlife origin or has an epidemiologically important wildlife host (Jones et al., 2008). Wild animals are susceptible to infection by many of the same pathogens that afflict domestic animals, and transmission between domestic animals and wildlife can occur in both directions. Nevertheless, the original event was often the transmission of a domestic animal disease to wildlife (Dobson and Foufopoulos, 2001). One such pathogen is *Mycobacterium bovis*, the causative agent of tuberculosis in cattle and most other mammals, wild or domestic. Importantly, its remarkably broad host range

includes humans (Grange, 2001). In 1900, tuberculosis was the leading cause of death in the United States. It is estimated that approximately 10% of all human tuberculosis cases were the result of exposure to cattle or cattle products (Olmstead and Rhode, 2004a). In 1917, *M. bovis* was responsible for approximately 15 000 deaths in the United States; three-times the number dying from all foodborne illnesses today (Olmstead and Rhode, 2004b). In most developed countries, public health concern over this zoonotic disease prompted officials to mandate milk pasteurization, as well as initiate nationwide cattle tuberculosis eradication programmes (Palmer and Waters, 2011). In most cases, eradication efforts have successfully decreased the incidence of bovine tuberculosis as well as *M. bovis* infection in humans. Notwithstanding, in some areas,

eradication has not been possible due to the presence of *M. bovis*-infected wildlife and wildlife-to-cattle transmission. As the prevalence of bovine tuberculosis in cattle decreases, the relative importance of *M. bovis*-infected wildlife increases and disease control measures are required for both livestock and wildlife. An understanding of the role wildlife play in the epidemiology of *M. bovis* infection is required.

When a pathogen reaches a certain prevalence in one species, and then spills over into other host species, it is known as 'pathogen spillover' or 'spillover effect' (Daszak et al., 2000). The recipient host species may be known as a 'spillover' host, while the source species may be known as a 'maintenance' or 'reservoir' host (Power and Mitchell, 2004). These definitions adequately describe host populations in general terms; however, in some situations, multiple wildlife species are involved, and each species may play a different role at the wildlife–domestic animal interface. These roles may differ with different pathogens. Consequently, the definition of terms such as 'reservoir host' are many, and in some cases are confusing, conflicting or incomplete (Haydon et al., 2002).

To aid our understanding of reservoir hosts of *M. bovis* definitions are required. It is important to define the population of interest or *target* population (Haydon et al., 2002). In many countries, and in the context of this review, cattle will generally be considered the target population. Any population that directly transmits *M. bovis* to the target population (i.e. cattle) is considered a *source* population (Haydon et al., 2002). In a given population, maintenance of *M. bovis* infection can be achieved only when sufficient intraspecies transmission exists to sustain disease at a threshold level. Epidemiologically, this level is described by the *basic reproduction rate* R_0 ('R naught'), which is defined as the expected number of secondary cases of infection produced in a population by a single infected animal (Diekmann et al., 1990; Renwick et al., 2007). The threshold for disease maintenance is met when $R_0 \geq 1$ (i.e. each infected animal transmits disease to one or more individuals). If $R_0 \geq 1$, the population is considered a *maintenance* host. If $R_0 < 1$, disease maintenance cannot be attained, and the population is a *non-maintenance* host (Caley and Hone, 2005). The R_0 is related to both the transmissibility of the pathogen as well as the size and density of the population. Population size can also be described using the epidemiological concept of *critical community size* (CCS). This is defined as the minimum size of a closed population within which a pathogen can persist indefinitely. In smaller populations, the number or density (i.e. prevalence) of infected hosts falls to low levels, random extinction is inevitable, and the pathogen cannot persist (Haydon et al., 2002). Populations smaller than CCS, or populations effectively made smaller than CCS through

control efforts, are non-maintenance populations. Pathogens will persist in populations larger than CCS where a sufficient number or density of infected hosts is present for pathogen persistence; consequently, these are maintenance populations (Haydon et al., 2002). In short, using both descriptors, maintenance host populations are those with $R_0 \geq 1$, or above CCS. Non-maintenance host populations are those with $R_0 < 1$, or below CCS. It should be noted that thresholds such as R_0 and CCS are not abrupt. They are also difficult to measure and may ignore many factors relevant to wildlife hosts such as seasonal births, seasonal variations in transmission and environmental or behavioral variations (Lloyd-Smith et al., 2005; Roberts, 2007).

Most developed countries where *M. bovis*-infected cattle are found have bovine tuberculosis eradication programmes. General practice is to test for cattle infected with *M. bovis* and remove infected animals. Consequently, in this context, cattle will be defined as a non-maintenance population. When a target population is smaller than CCS, it cannot maintain pathogen presence (i.e. cattle, a non-maintenance host). In such a case, completely isolating the target population from outside transmission sources should cause the pathogen to become extinct in the target population (Haydon et al., 2002). A reservoir is present if the pathogen repeatedly appears in such an isolated non-maintenance target population (Haydon et al., 2002). Therefore, a more precise definition of *reservoir* host is one or more epidemiologically connected populations or environments in which the pathogen can be permanently maintained, and from which infection is transmitted to the defined target population. In other terms, a reservoir host is a maintenance host from which disease spills over into a target population. As such, reservoirs may be a single host population or several host populations, that when combined constitute a 'community' host population (Haydon et al., 2002).

It is generally accepted that wildlife reservoir hosts of *M. bovis* include the brushtail possum (*Trichosurus vulpecula*) in New Zealand, European badger (*Meles meles*) in Great Britain and Ireland, African buffalo (*Syncerus caffer*) in South Africa, wild boar (*Sus scrofa*) in the Iberian Peninsula and white-tailed deer (*Odocoileus virginianus*) in Michigan, USA. Related to the conceptual epidemiological ideas of R_0 and CCS, and discussed specifically in terms of *M. bovis* infection by Delahay et al. (2001), are three important tangible disease/pathogen characteristics used to identify *M. bovis* reservoir host populations: (i) disease prevalence, (ii) *M. bovis*-induced pathology and (iii) host ecology and behaviour. Using examples from known or potential reservoir hosts of *M. bovis*, an examination of both the conceptual epidemiological characteristics of R_0 and CCS, as well as the more evident disease/pathogen characteristics will be illustrative.

Disease Prevalence

Prevalence not only refers to the number of infected animals in a given population, but also the location of infected animals, both geographically and temporally (Delahay et al., 2001). The idea of disease prevalence is broadened through the concept of CCS and R_0 . In populations below CCS or where $R_0 < 1$, disease prevalence will eventually decrease to zero and result in pathogen extinction. Importantly, prevalence is generally not uniform over large areas. In some cases, clusters of high prevalence exist. High-prevalence clusters may be viewed as multifocal areas where CCS is achieved or $R_0 \geq 1$. Critical community size and R_0 may be higher in clusters compared with surrounding areas due to habitat preference, availability of food or anthropogenic factors such as fences, roads and supplemental feeding sites (Miller and Kaneene, 2006). High-prevalence clusters also illustrate the important point that CCS and R_0 are not static, but change with changing conditions making calculation difficult (Lloyd-Smith et al., 2005).

In the United States (US) prior to 1994, prevalence of tuberculosis in white-tailed deer was unknown, but probably exceedingly low. In 1975, a 'hunter-killed' white-tailed deer in northeast Michigan was diagnosed with tuberculosis due to *M. bovis* (Schmitt et al., 1997). Follow-up surveys to identify other infected deer were not conducted. It is likely that at that time, white-tailed deer were assumed to be a non-maintenance host (i.e. below CCS or $R_0 < 1$). In the setting of early to mid-1900s in the United States, unlike today, cattle were considered the source population, and deer were considered the target host. In the latter 1900s, Michigan's cattle tuberculosis eradication effort was progressing, and in 1979, Michigan was granted TB-free status, in regard to cattle, by the US Department of Agriculture (USDA). Continued monitoring for *M. bovis* infection and removal of any tuberculous cattle, rendered Michigan cattle a non-maintenance host population. Cattle would now be considered a target population.

In 1994, another 'hunter-killed' deer was identified with tuberculosis due to *M. bovis*. This deer was found only 13 km from the site where the tuberculosis deer was identified in 1975. Subsequent surveys of deer in the area identified a focus of *M. bovis* infection in white-tailed deer in northeast Michigan (Schmitt et al., 1997). Unknown to all, increasing tuberculosis prevalence in a growing population of white-tailed deer had reached a threshold where CCS was achieved or $R_0 \geq 1$. White-tailed deer had become a maintenance population and reservoir of *M. bovis*. Several factors are thought to have contributed to the establishment and persistence of *M. bovis* in Michigan deer. It is postulated that *M. bovis* was transmitted from cattle to deer during the early 1900s when

the prevalence of tuberculosis in Michigan cattle was high (Frye, 1995). During this period, cattle were a maintenance population and potential source of *M. bovis* for sympatric deer. One model suggests *M. bovis* spilled over from Michigan cattle to deer as early as the 1950s (McCarty and Miller, 1998).

Historically, deer density in northeast Michigan has been higher than that measured in other regions of the state. The presence of private 'hunt clubs' in this region, that occupied large geographic areas where supplemental feeding and restricted hunting were regularly practiced, allowed for deer densities to reach levels as high as 31 deer/km². Contrastingly, the average in surrounding areas was approximately 5 deer/km² (O'Brien et al., 2006). Importantly, areas of historically high deer density corresponded to current areas of highest tuberculosis prevalence in deer. These areas also corresponded to regions of highest tuberculosis prevalence in cattle prior to granting of TB-free status in 1979. During the early 1900s, northeast Michigan had bovine tuberculosis reactor rates of 20–30% (Miller and Kaneene, 2006; O'Brien et al., 2006). Through the 1950s, Michigan cattle accounted for 30% of all tuberculous reactors in the United States (Miller and Kaneene, 2006). At that time, the cattle population acted as a source of *M. bovis* for wild deer. It would be assumed therefore that in areas of both high deer density and high cattle tuberculosis rates, one or more spill-over events from cattle to deer occurred, CCS was achieved or $R_0 \geq 1$.

Control efforts within the affected region have decreased deer density through increased hunting and restrictions on supplemental feeding. These efforts have successfully decreased apparent prevalence from 4.9% to <2% (O'Brien et al., 2006). Nevertheless, clusters of higher prevalence still exist. Clusters of highest prevalence occur almost exclusively on private land where the practice of supplemental feeding has been the greatest in magnitude and duration (O'Brien et al., 2006). One could assume that practices such as supplemental feeding and restricted hunting, created foci of increased deer density where CCS was achieved or R_0 achieved a value ≥ 1 .

Although it is postulated that Michigan deer contracted *M. bovis* from Michigan cattle (i.e. spillover from cattle to deer), transmission occurs in both directions. Since 1995, there have been 57 Michigan cattle herds identified as *M. bovis*-infected. Most infected herds have been located in the same region of northeast Michigan as most of the tuberculous deer, including the deer identified in 1975 and 1994. Infected cattle herds have not been identified in other regions of Michigan. Today in northeast Michigan, white-tailed deer represent a *M. bovis* maintenance population epidemiologically connected to tuberculous cattle via deer-to-cattle transmission. By

definition, deer in northeast Michigan are a *M. bovis* reservoir host population. It is, however, important to note that species alone does not designate reservoir host assignation, as the following example from Minnesota, USA illustrates.

In 2005, in Minnesota, considered TB-free by the USDA since 1971, the identification at an abattoir of a tuberculous beef cow from northwest Minnesota prompted an epidemiological investigation. The investigation revealed multiple *M. bovis*-infected cattle herds, all in northwest Minnesota. The investigation included sampling of white-tailed deer in areas near infected herds (Carstensen and Doncarlos, 2011). Sampling of deer determined the apparent prevalence was 0.2%, much lower than that seen in northeast Michigan. The last *M. bovis*-infected deer was found in 2009, and the last infected cattle herds were found in late 2008. By 2011, the Minnesota Department of Natural Resources (MNDNR) had removed over 9000 deer, through culling and liberalized hunting, thus decreasing community size. Sampling of deer and cattle in 2010, 2011 and 2012 failed to reveal additional tuberculous deer or cattle. In contrast to Michigan, Minnesota white-tailed deer did not represent a reservoir. It is apparent that white-tailed deer were epidemiologically connected to the target cattle population and able to transmit *M. bovis* to Minnesota cattle. However, they were unable to maintain infection (i.e. non-maintenance population), as prevalence was too low, suggesting that the population was below CCS or $R_0 < 1$.

The contrasting situations in Michigan and Minnesota reveal some instructive comparisons. In both Michigan and Minnesota, spillover from cattle to deer is highly likely given the similarities, in each respective state, of cattle and deer isolates by RFLP, variable number tandem repeat (VNTR) and other molecular comparisons (Whipple et al., 1997; Miller and Kaneene, 2006). Following spillover from cattle in Michigan, a reservoir of *M. bovis* formed in white-tailed deer. This was probably due to factors such as multiple spillover events, high deer density and unnatural congregation of deer around supplemental feeding sites, thus creating multifocal areas where CCS was achieved or $R_0 \geq 1$ (i.e. clusters of higher prevalence). In contrast, in Minnesota, deer density was consistently lower, supplemental feeding was not widely practiced, and fewer tuberculous cattle were present, resulting in fewer spillover events. In this environment prevalence remained low, CCS was not achieved, or deer-to-deer transmission was insufficient (i.e. $R_0 < 1$) for disease maintenance (Carstensen and Doncarlos, 2011). The contrasting situations in Michigan and Minnesota illustrate the role of the measurable characteristic of disease prevalence, as well as the less tangible concepts of CCS and R_0 in the establishment, or non-establishment, of a reservoir host population.

Pathology

Mycobacterium bovis-induced pathology defines disease course, as well as the potential for excretion and transmission of viable (i.e. infectious) *M. bovis*, which directly impacts R_0 . Likewise, the character and severity of lesions influence the rate of mortality, which directly impacts community size. Together, disease course, excretion potential and mortality rate dictate pathogen output and duration of the infectious period (Delahay et al., 2001; Corner, 2006). Pathology resulting from *M. bovis* infection is the direct result of factors such as dose and route of infection. Among reservoir hosts, higher doses of *M. bovis* are associated with more severe pathology and a more rapid course of disease. In susceptible species, the dose required to establish infection and disease via aerosol exposure is believed to be low, in some cases, only a few bacilli. In contrast, much higher doses are required to establish infection and produce disease via the oral route (Francis, 1947). Therefore, the same species exposed to different doses of *M. bovis* through different routes may display different patterns and severity of lesions (i.e. pathology).

Spain, a major livestock producer within the European Union, has over 6 million cattle in over 140 000 herds (Gortazar et al., 2011). Bovine tuberculosis eradication efforts have decreased the prevalence from 12% in 1987 to 1.68% in 2008. In Portugal, incidence of tuberculosis in cattle was 0.22% in 2008 with 99.3% of the more than 70 000 Portuguese cattle herds declared as ‘officially free of tuberculosis’ (Santos et al., 2009). In Spain, there remain multiple regions of higher cattle tuberculosis prevalence. These are generally regions where habitat is shared by cattle and wildlife (Gortazar et al., 2011). Additionally, in southwestern Spain, there are 2.5 million domestic pigs raised in open habitats where interactions with wildlife occur (Gortazar et al., 2011).

Wild boar are the most widely distributed free-ranging ungulate in the Iberian Peninsula (Martin-Hernando et al., 2007). Additionally, in many areas, wild boar are managed for hunting purposes on private estates (Vicente et al., 2006). In Portugal, rather than hunting estates, wild boar are primarily associated with historic refuges where they have always been present (Santos et al., 2009). One ecosystem where wild boar are not managed, and thereby less influenced by anthropogenic activities such as fencing, feeding and limited genetic heterogeneity, is the Doñana Biosphere Reserve in southern Spain (Gortazar et al., 2008). Surveys of wild boar in the Doñana Reserve and Mediterranean Spain show the paired mandibular lymph nodes are the most commonly affected tissues (> 90%) (Martin-Hernando et al., 2007), suggesting a primary oral route of infection. Generalized disease (i.e. lesions in > 1 anatomic region) can be seen in > 50% of tuberculous

boar, most commonly juveniles (Vicente et al., 2006; Martin-Hernando et al., 2007). Animals with severe and generalized disease have a higher excretion potential via multiple routes (e.g. aerosolized respiratory excretions, saliva), increasing the likelihood of transmission and increasing R_0 .

In both countries, molecular analyses, including spoligotyping and VNTR typing, demonstrate isolates that are shared by wild boar, cattle (Gortazar et al., 2005; Hermoso de Mendoza et al., 2006; Cunha et al., 2012) and domestic pigs (Parra et al., 2003). Therefore, it has been demonstrated in the Iberian Peninsula that wild boar are a population epidemiologically connected to the target hosts of both cattle and domestic pigs. In both Spain and Portugal, high *M. bovis* infection rates (approximately 50%) are found in wild boar populations not associated with cattle or other probable hosts of *M. bovis* (e.g. cervid species) (Parra et al., 2003; Vicente et al., 2006), suggesting *M. bovis* can be maintained in Iberian wild boar. Therefore, by definition, wild boar in the Iberian Peninsula can be considered a *M. bovis* reservoir, as they are a maintenance host with epidemiological connections to target hosts (cattle and domestic swine) (Naranjo et al., 2008).

In contrast to Iberian wild boar, feral hogs in Australia's Northern Territory presented with *M. bovis*-induced pathology described as localized (i.e. a single location or anatomic region) in > 80% of pigs (Corner et al., 1981). Primary involvement of the mandibular lymph nodes suggested that the primary route of transmission was oral. Only 6.7% displayed a pattern of generalized disease and pulmonary involvement was uncommon (Corner et al., 1981). Due to limited pathological effects and the absence of both pulmonary lesions and generalized disease, the excretion potential among Australian feral hogs was quite low. The characteristics of limited lesion development (i.e. *M. bovis*-induced pathology), low excretion potential and a single route of exposure are conditions compatible with a $R_0 < 1$ and consistent with studies declaring feral hogs in Australia's Northern Territory not to be a reservoir host population of *M. bovis* (Corner et al., 1981). Indeed, after the removal of tuberculous cattle and buffalo (*Bubalus bubalis*) from Australia's Northern Territory, prevalence in feral hogs decreased from 19% in 1981 to 0.25% in 1992 (Corner et al., 1981; McInerney et al., 1995). It is apparent that, in contrast to Iberian wild boar, limited pathology in *M. bovis*-infected feral hogs contributed to a low excretion potential and low $R_0 < 1$. Once the source of *M. bovis* (i.e. cattle, wild buffalo) was removed, feral hogs were unable to maintain pathogen presence; therefore, they are a non-maintenance host and not a *M. bovis* reservoir.

Although both Iberian wild boar and Australian feral hogs are taxonomically *Sus scrofa*, observed differences in *M. bovis*-induced pathology could also be influenced by differences in phylogeny and host genetic susceptibility.

Indeed, molecular analyses of phylogenetic relationships demonstrate that present-day Australian feral hogs are of both European and Asian origin (Kim et al., 2002; Gongora et al., 2004). Iberian wild boar, in contrast, appear to be descendants of European domestic pigs (Alves et al., 2003). Moreover, genetic analysis of wild boar in Spain demonstrates genetic-related resistance and susceptibility. Animals with high genetic heterozygosity are more capable of resisting infection and controlling disease progression (Acevedo-Whitehouse et al., 2005).

Host ecology and behaviour

Host ecology and behaviour dictate the extent to which an infected host brings itself or its excretions into close contact with other susceptible hosts (Delahay et al., 2001), including members of the same species (i.e. intraspecies transmission). These factors are likely to directly influence CCS and R_0 . For example, the natural denning behaviour (Day et al., 2000) of the brushtail possum and badger provide an opportunity for close intraspecies contact, increasing the probability of either direct (aerosol) or indirect (environmental contamination) transmission.

Social organization also influences intraspecies contact, the potential spread of disease, CCS and R_0 . The social structure of white-tailed deer is characterized by semi-permanent family groups composed of a matriarchal doe, one to several generations of her female offspring, and their respective fawns. Offspring of both genders remain with their dam until she approaches parturition the following year, at which time they are forced out of the family group. After parturition, yearling does, with or without fawns, rejoin their respective dams, now with suckling fawns at their sides. Yearling males do not rejoin the family group (O'Brien et al., 2002). The intimate interactions between doe and fawn, as well as among all members of these matrilineal groups, increase the likelihood of both direct and indirect transmission of *M. bovis* and positively influence R_0 . Indeed, genetic relatedness was higher among *M. bovis*-infected deer than non-infected deer in the same location (Blanchong et al., 2007). It is important to note that genetic resistance and susceptibility could also explain such observations. In fact, such resistance or susceptibility to *M. bovis* infection has been demonstrated in red deer (*Cervus elaphus*) in New Zealand (MacKintosh et al., 2000, 2011).

Normal behaviour can be altered by anthropogenic activities such as fencing and supplemental feeding (O'Brien et al., 2011). A behaviour or ecological trait that promotes disease transmission within a host population will increase R_0 . For example, behaviours that put members of a population in close proximity to one another (i.e. familial groups, supplemental feeding sites) will increase the R_0 (Diekmann

et al., 1990; Renwick et al., 2007). Similarly, the number or density of infected hosts in a closed population defines CCS (Haydon et al., 2002). Behaviours such as familial groups, denning or gathering at supplemental feeding sites increase both number and density and aid in achieving CCS.

Multihost systems

In several countries with suspected or confirmed wildlife reservoirs of *M. bovis*, multiple species are epidemiologically linked and may include multiple reservoir hosts and multiple routes of transmission (Caley and Hone, 2005). Moreover, each species may differ in the epidemiological characteristics of R_0 and CCS as well as disease/pathogen characteristics of prevalence, pathology, ecology and behaviour (Power and Mitchell, 2004). These relationships create complex patterns of intra- and inter-species transmission (Nugent, 2011), as the following examples illustrate.

In the Iberian Peninsula, a multihost system of *M. bovis* includes wild boar, red deer, cattle and domestic pigs. In various surveys, an extraordinary proportion of both red deer and wild boar have presented with disseminated tuberculosis involving numerous organs (Vicente et al., 2006; Gortazar et al., 2008). Wild boar routinely scavenge carrion (e.g. dead tuberculous deer), which may explain much of the deer-to-boar transmission (Vicente et al., 2007; Gortazar et al., 2008). Efficient scavengers, consuming highly infectious material, create an ideal condition for disease transmission. In such cases, red deer are acting as a source of *M. bovis* for wild boar.

Wild boar are implicated as a reservoir of *M. bovis* for the target hosts of cattle and domestic pigs. The role of red deer is less clear. The literature on New Zealand red deer as a *M. bovis* maintenance and reservoir host population is substantial (Lugton et al., 1997, 1998; de Lisle et al., 2001). However, the literature on red deer in Spain is less extensive. Spanish red deer may be maintenance or non-maintenance host populations and therefore may or may not be a reservoir population. It is also possible that together, as a reservoir community (Haydon et al., 2002), wild boar and red deer constitute a source of *M. bovis* for cattle and pigs. It is clear that *M. bovis* strains from red deer and wild boar in Spain share similar spoligotypes, suggesting transmission between wild boar and deer (Gortazar et al., 2005; Hermoso de Mendoza et al., 2006). Both wild boar and red deer congregate at watering and feeding sites, aiding the achievement of both CCS and R_0 , and there is strong evidence of inter-species transmission at such gathering sites (Vicente et al., 2007). Gathering at these sites significantly increases the risk of tuberculosis in both species (Vicente et al., 2007). Models have shown that inter-species transmission at watering and feeding sites is more likely to be from boar-to-deer rather than from

deer-to-boar (Vicente et al., 2007). The precise reason for this difference is unknown; however, there may be several contributing factors. At managed feeding and watering sites, feeders containing supplemental feed for red deer are raised above the ground, making access by wild boar difficult. It also makes deer-to-boar transmission through shared feed improbable. Contrastingly, feed for wild boar is often scattered on the ground or placed in feeders low to the ground, and easily accessed by both boar and deer, making indirect transmission through shared feed or contaminated soil a potential means of boar-to-deer transmission (Vicente et al., 2007). Scavenging of infected carcasses is a likely mechanism of deer-to-boar transmission, but not boar-to-deer transmission. A critical question relevant to the host status of red deer in Spain is whether in the absence of sympatric *M. bovis*-infected wild boar, red deer can maintain pathogen presence. That is to say, are red deer a maintenance host population and potential reservoir? One study examined both inter-species and intraspecies transmission in red deer and wild boar and failed to find significant relationships consistent with intraspecies transmission among red deer at feeding sites (Vicente et al., 2007). This finding is consistent with a $R_0 < 1$. In the multihost system of Spain, transmission occurs between wild boar and red deer. In addition, wild boar are a source of *M. bovis* for livestock (i.e. cattle and domestic swine); however, red deer in Spain appear to be a non-maintenance, non-essential population in Spain's multihost system. Accordingly, one would expect, if *M. bovis* were eliminated from wild boar, *M. bovis* infection would eventually disappear from red deer in Spain.

The number and diversity of species infected by *M. bovis* make the multihost system of South Africa perhaps the most complex. In South Africa, there are at least two accepted reservoir hosts [African buffalo and greater kudu (*Tragelaphus strepsiceros*)], and at least twelve other recognized hosts, presumed to be non-maintenance hosts. (Kalem-Zikusoka et al., 2005; Michel et al., 2006; Renwick et al., 2007). In South Africa's Kruger National Park (KNP), buffalo were first diagnosed with tuberculosis due to *M. bovis* in 1990 (Bengis et al., 1996). Circumstantial, as well as molecular epidemiological evidence, suggests cattle herds adjacent to the southern boundary of KNP were the original source of *M. bovis*, and that spillover from cattle to buffalo occurred during the 1950s and 1960s when buffalo leaving the park grazed with sympatric *M. bovis*-infected cattle (Bengis et al., 1996; Michel et al., 2009). During this time, control measures were initiated to eliminate tuberculosis from cattle herds, but tuberculosis remained unknown and undetected in KNP buffalo until 1990. Spread of tuberculosis among the Kruger buffalo population has been relatively rapid. In 1992, the prevalence of tuberculosis in northern, central and southern zones of KNP was 0, 4.4% and 27.1%, respectively. By 1998, the prevalence had

increased to 16% and 38.2% in the central and southern zones, respectively (Michel et al., 2006). *Mycobacterium bovis* infection in buffalo was diagnosed 40 km south of the northern park border (Michel et al., 2006) and 45 km north of the park border in Zimbabwe (de Garine-Wichatitsky et al., 2010) in 2004 and 2008, respectively. This rate of spread, rapid in terms of tuberculosis, suggests that $R_0 \geq 1$; that is, on average, each infected buffalo transmits disease to at least one, and more likely several other buffalo. *Mycobacterium bovis*-induced pathology in buffalo is progressive and not well encapsulated (De Vos et al., 2001), meaning pulmonary lesions spread throughout the lung, including bronchi and bronchioles, increasing the likelihood of highly infectious respiratory aerosols.

Disease persistence and geographic expansion also suggest that herds of buffalo in KNP are above CCS. Behaviorally, buffalo are highly gregarious, living in large herds of 200–1000 animals in KNP (De Vos et al., 2001; Michel and Bengis, 2012). Buffalo-to-buffalo transmission is probably via aerosol. Aerosol transmission, combined with social reorganization (i.e. fission and fusion) of herds takes place regularly, enhancing disease spread and positively influencing R_0 (Halley et al., 2002; Cross et al., 2005).

Thus far, cattle have been viewed as the target host, where the implications of *M. bovis* wildlife reservoirs are ultimately of agricultural economic importance due to loss of trade opportunities, increased testing costs, decreased production, etc. However, viewing valuable endangered or vulnerable native wildlife as target populations is also valid. Tuberculosis in these species impacts conservation and wildlife-based tourism, as well as public acceptance of animals moving across borders in large multinational trans-frontier conservation areas (Michel et al., 2006).

One target population with important conservation implications is the African lion (*Panthera leo*). Buffalo are one of the preferential prey of lions, so in regions where tuberculosis is present in buffalo, there is a notable prevalence of tuberculosis in lion prides. Transmission from buffalo to lion is probably the result of repeated oral exposure to large amounts of *M. bovis*-infected buffalo tissue (Keet et al., 1996; Trinkel et al., 2011). With some exceptions, *M. bovis*-induced pathology in non-maintenance hosts is limited (Bruning-Fann et al., 1998, 2001; Palmer et al., 2002). Contrastingly, in lions, *M. bovis* induces severe pathology characterized by pneumonia, emaciation, non-healing wounds, lameness and blindness (Keet et al., 1996; Michel et al., 2006). Multiple organ systems are affected suggesting multiple routes of infection followed by haemogenous or lymphatic dissemination. Microscopically, in contrast to typical *M. bovis*-induced pathology, lesions in lions are extensive and proliferative but without significant necrosis. Mortality often results from tuberculosis in lions. Small and isolated populations are especially vulnerable

due to lack of heterogeneity from inbreeding, leaving them more susceptible to disease (Trinkel et al., 2011).

It is unclear whether lions represent a non-maintenance or maintenance host (Michel et al., 2006). Some report that in regions of high disease prevalence in buffalo, there is a spatial spread of *M. bovis* infection within lion prides in the same region (Michel et al., 2006). A separate study failed to find a positive correlation between the prevalence of lion tuberculosis and that of buffalo (Ferreira and Funston, 2010). On this point rests a critical question in determining reservoir host status, for if disease prevalence in lions is driven by disease prevalence in buffalo, rather than intraspecies transmission, it suggests that lions are non-maintenance hosts (Renwick et al., 2007). On the other hand, extensive lesion formation including granulomatous bronchopneumonia and granulomatous enteritis (Keet et al., 2010) suggests that respiratory exudates and faeces would be probable routes of bacterial excretion. Moreover, sociality and aggressive intraspecies interactions are behaviours that should enhance lion-to-lion transmission and promote disease maintenance (Michel et al., 2006). The R_0 of tuberculosis in lions is unknown, and the true significance of *M. bovis*-induced pathology and social behaviour is unclear. In spite of some characteristics that would suggest possible maintenance host potential, to date, there is no strong supportive data to categorize lions as reservoir hosts of *M. bovis* (Renwick et al., 2007).

Another suspected reservoir host of *M. bovis* in the South African multihost system is the greater kudu. A common manifestation of clinical *M. bovis* infection in kudu is abscessation and fistulation of cranial lymph nodes, most commonly the parotid nodes (Bengis et al., 2001). This characteristic of *M. bovis*-induced pathology plays an integral part in the suspected epidemiology. It is believed infectious exudates from draining fistulae contaminate thorns and leaves, such as that of *Acacia* spp. trees that are common browse for kudu. Other kudu are exposed to *M. bovis* by ingestion of contaminated browse or through percutaneous scratches from thorns (Renwick et al., 2007). In this case, characteristics of pathology (draining fistulae), behaviour (browse feeders, food preference) and host ecology (presence of thorny *Acacia* spp.) are all important. This mechanism may explain intraspecies transmission and present a means by which R_0 can achieve a value ≥ 1 . The means by which inter-species transmission with buffalo occurs is less clear. Genotypic analysis shows some *M. bovis* isolates from kudu and buffalo to be the same; however, a strain of *M. bovis* seemingly specific for kudu has also been identified (Renwick et al., 2007; Michel et al., 2009). Not found in other species, a ‘kudu-specific’ *M. bovis* strain could help explain how *M. bovis* is maintained in kudu.

Other roles for hosts in multihost systems

In multihost systems, different species may play different roles. In New Zealand, there are at least four epidemiologically important hosts, brushtail possums, ferrets (*Mustela furo*), red deer and feral pigs (Coleman and Cooke, 2001; Nugent, 2011). Brushtail possums are considered the primary reservoir for disease in cattle, while ferrets, red deer and wild pigs are generally regarded as spillover hosts (Nugent, 2011). However, under certain conditions, ferrets and red deer may be considered reservoirs (Caley and Hone, 2005).

Red deer can live up to 20 years of age, much longer than the possum. By virtue of their longevity, deer may serve as temporal vectors (i.e. carry *M. bovis* through time) (Nugent, 2011). Deer infected while young may remain latently infected for many years beyond the normal possum lifespan. The ramifications of which is that possum and deer control efforts need to continue for several years after tuberculous possums have been removed, as infected deer may still represent a potential source of infection for remaining or immigrating possums (Nugent, 2011).

In other cases, intraspecies transmission may be just below the threshold required for disease persistence (i.e. $R_0 < 1$). A population where $R_0 < 1$ does not mean it is an inconsequential host species, especially if R_0 is close to 1 (e.g. $R_0 = 0.75$) (Dobson and Foufopoulos, 2001; Caley and Hone, 2005). In such a population, there are a substantial number of secondary infections, but too few to maintain disease. Only occasional inter-species transmission from a reservoir host is required for the sum of intra- and inter-species transmissions to be great enough such that maintenance may be achieved or spillover to a third population may occur (Nugent, 2011).

Some species with large home ranges can serve to geographically disperse disease. For example, in some regions of New Zealand, feral hogs are considered spillover hosts, contracting disease as they feed on tuberculous possum carcasses. Feral hogs have larger home ranges than possums; therefore, as tuberculous pigs travel to the limits of their home range and die, non-infected possums may feed on pig carcasses, thereby successfully transmitting disease over a wider geographic area than possum-to-possum transmission would allow (Nugent, 2011).

Disease Control in Wildlife Reservoirs

Transmission of *M. bovis* across the wildlife–domestic animal interface represents a significant obstacle to bovine tuberculosis eradication in several countries (e.g. New Zealand, Great Britain and Ireland, US) (Corner, 2006). In spite of long-standing, expensive and somewhat successful efforts over many decades, animal health officials have

found that traditional test and slaughter methods, the centrepiece of most bovine eradication programmes, are of limited success when affected cattle herds have contact with infected wildlife. Two methods described for dealing with tuberculosis and other diseases in wildlife include: (i) limiting the number of receptive and infected individuals by culling (lethal control) or vaccination, and (ii) decreasing the number of infected animals through treatment or selective test and cull practices (Artois et al., 2011). Population thresholds (i.e. R_0 and CCS) are the foundation for efforts to eradicate wildlife diseases by reducing the number of susceptible hosts (Lloyd-Smith et al., 2005). The goal of lethal control is to reduce the density of both susceptible and infected animals in a population. Decreased intraspecies transmission will eventually reach a point where population size is smaller than CCS, $R_0 < 1$ and the disease is extinguished (Artois et al., 2011). Such lethal control efforts have been conducted in Ireland (Corner et al., 2011), the United States (O'Brien et al., 2006), Great Britain (Donnelly et al., 2007), New Zealand (Caley et al., 1999) and elsewhere, with variable results (Donnelly et al., 2006; O'Brien et al., 2006). One of the most controversial exercises involving both lethal control (*proactive culling*) and selective test and cull (*reactive culling*) is in the United Kingdom (UK). The UK badger culling experience also represents an example of the role of behaviour and ecology in terms of *M. bovis* reservoir host species.

In 1998, a large experiment was implemented known as the randomized badger culling trial (RBCT). The RBCT was designed to determine the role of badgers as a reservoir of *M. bovis* and to compare the effects of three different control strategies: no culling of badgers, localized selective culling of badgers in response to identified cases of tuberculosis in cattle (reactive culling), and removal of all badgers across trial areas (proactive culling). The trial clearly demonstrated that infected badgers were a reservoir of infection for cattle (Bourne et al., 2006). Reactive culling was prematurely curtailed when analysis suggested that reactive culling had increased disease risk in cattle herds (Donnelly et al., 2003). In contrast, after 5 years of proactive culling, there was a 23% reduction in the incidence of cattle tuberculin reactors inside the culling area (Wilson et al., 2011). Why did reactive culling result in an increased risk for tuberculosis in cattle? Badgers have complex social structures, the stability of which varies with population density (Cresswell and Harris, 1988). Examination of the RBCT culling areas revealed that culling resulted in altered behaviour. The changed behaviour resulted in social restructuring and increased home range of remaining badgers. Increased ranging behaviour probably resulted in increased contact with other badgers (i.e. an increase in R_0) as well as cattle (Vial and Donnelly, 2012).

Table 1. Disease and pathogen characteristics of *Mycobacterium bovis* wildlife reservoir hosts and their relationship to the epidemiological concepts of basic reproduction rate (R_0) and critical community size (CCS)

Disease/Pathogen characteristic	Definition	Relationship to R_0 and CCS	Selected examples	Selected references
Prevalence	Number or density of infected animals in a given population.	High prevalence is associated with $R_0 \geq 1$ and CCS above threshold level.	Wild boar in Spain Wtd in Michigan, USA	Gortazar et al. (2011) Schmitt et al. (1997) O'Brien et al. (2006)
<i>Mycobacterium bovis</i> -induced pathology	Nature of lesions (solid, caseous, liquefactive) as well as lesion distribution (number of anatomic sites with lesions).	Pathology defines excretion potential, impacting R_0 . Generalized, necrotic lesions enhance transmissibility and R_0 . Disease severity effects mortality and alters community size.	Wild boar in Spain African buffalo	Vicente et al. (2006) Gortazar et al. (2008) De Vos et al. (2001) Keet et al. (2010)
Host ecology/behaviour	Behaviours or ecological traits that affect disease transmission.	Artificial feeding results in multifocal increases in density, increasing community size and transmission (R_0). Behaviours and ecological traits that draw animals together will influence transmission (R_0) and community size.	Supplemental feeding of wtd in USA and wild boar and red deer in Spain. Social structure of wtd. Social structure of Eurasian badger.	Vicente et al. (2007) Miller and Kaneene (2006) Blanchong et al. (2007) Vial and Donnelly (2012)

wtd, white-tailed deer.

Complete removal of any wildlife reservoir of infection would be extremely difficult and unethical. A non-lethal method drawing much attention over the last decade is wildlife vaccination. An effective tuberculosis vaccine for wildlife need not provide sterile immunity. In point of fact, in some cases, infection need not be prevented if *M. bovis*-induced pathology can be reduced or minimized. Highly necrotic lesions often contain many more bacilli than non-necrotic lesions (Palmer et al., 2007). Highly necrotic lesions in lungs, intestinal tracts or superficial lymph nodes are more likely to expand and enter portals of excretion such as airways, intestines or external draining fistulae, enhancing excretion potential and R_0 .

Wildlife vaccines for tuberculosis, as well as vaccine delivery methods have been described in New Zealand, Great Britain, Ireland, Spain and the United States. Most strategies involve the use of *M. bovis* bacillus Calmette–Guérin (BCG), the only approved TB vaccine for humans (Al-dowell et al., 1995; Ballesteros et al., 2009; Palmer et al., 2009; Carter et al., 2012). BCG induces protection in various species (brush-tail possum, European badger, Eurasian wild boar, white-tailed deer) after being administered by subcutaneous, conjunctival, intranasal and intramuscular routes (Corner and Buddle, 2005; Nol et al., 2008; Ballesteros et al., 2009; Palmer et al., 2009; Carter et al., 2012). Another non-lethal method of control is treatment of animals with antituberculosis drugs. This is generally considered impracticable, but has been used with individual animals in zoological collections. (Duncan et al., 2009; Dumonceaux et al., 2011).

Conclusions

This review discussed characteristics used to define a *M. bovis* wildlife reservoir. Examination may utilize epidemiological concepts such as R_0 and CCS. Although useful in a conceptual manner, R_0 and CCS are difficult to measure. More tangible characteristics to examine are disease/pathogen characteristics such as, (i) disease prevalence, (ii) *M. bovis*-induced pathology and (iii) host ecology and behaviour. However, in the context of *M. bovis* reservoir hosts, an approach, as presented here, that considers both conceptual and tangible factors is instructive (Table 1).

For example, the related characteristics of prevalence, CCS and R_0 are worthwhile consideration in the contrasting situations in Michigan and Minnesota. In these cases, dissimilar prevalence rates, which influence CCS, resulted in the establishment of a *M. bovis* reservoir in white-tailed deer in Michigan, but not in Minnesota. This situation also emphasizes the importance of wildlife surveys, and early detection of infected wildlife, in regions where cattle tuberculosis outbreaks occur.

Patterns of *M. bovis*-induced pathology determine excretion potential and are a critical aspect of R_0 , highlighting the importance of thorough post-mortem examination. The differing lesion characteristics in European wild boar and Australian feral hogs show that under different conditions, routes of excretion and infection may differ. R_0 is directly affected by excretion potential and route of infection.

It is imperative to examine host ecology and behaviour, with attention to anthropogenic influences (e.g.

supplemental feeding, habitat encroachment), as they may be more controllable than natural factors (e.g. denning, sociality). Behaviour, habitat preferences, social structure and environment influence the means by which infection is maintained and transmitted and are fundamental in the ascertainment of R_0 and CCS.

Complex multihost systems yield complicated inter-species interactions involving prevalence, pathology and behaviour between multiple potential reservoir and non-reservoir hosts. Moreover, in multihost systems, one must consider the possibility of multihost reservoir communities. The presence of pathogens in a multihost system with host preference, such as a 'kudu-specific' strain of *M. bovis*, demonstrates the possible role of pathogen dissimilarity in reservoir creation and maintenance.

The barriers between wildlife, livestock and humans are disappearing, and new challenges continue to arise in the management of livestock and wildlife. Transmission of some pathogens between livestock and wildlife can have enormous impact in terms of trade, public health and conservation (Briones et al., 2000; Michel et al., 2006, 2010). The need to determine *M. bovis* host reservoir status is not likely to decrease. It will be critical to collect data to determine prevalence and pathology, to define behaviour and ecology, and consider epidemiological concepts such as R_0 and CCS, not only to determine host status, but also to aid in determination of control efforts.

Conflicts of Interest

The author has no conflicts of interest to declare.

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