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REVIEW

Open questions and recent advances in the control of a multi-host infectious disease: animal tuberculosis

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ABSTRACT

1. Animal tuberculosis (TB) control is globally important for public health, economics and conservation. Wildlife species are often part of the *Mycobacterium tuberculosis* complex (MTC) maintenance community, complicating TB control attempts.
2. We describe the current knowledge on global TB distribution and the significance of wildlife hosts; identify insufficiently known aspects of host pathology, ecology and epidemiology; present selected time series in wildlife TB; and summarize ongoing research on TB control, providing additional insight on vaccination.
3. Six specific research needs are identified and discussed, namely: 1) complete the world map of wildlife MTC reservoirs and describe the structure of each local MTC host community; 2) identify the origin and behaviour of generalized diseased individuals within populations, and study the role of factors such as co-infections, re-infections and individual condition on TB pathogenesis; 3) quantify indirect MTC transmission within and between species; 4) define and harmonize wildlife disease monitoring protocols, and apply them in a way that allows proper population and prevalence trend comparisons in both space and time; 5) carry out controlled and replicated wildlife TB control experiments using single intervention tools; 6) analyse cost-efficiency and consider knowledge transfer aspects in promising intervention strategies.
4. We believe that addressing these six points would push ahead our capacities for TB control. A remaining question is whether or not interventions on wildlife TB are at all justified. The answer depends on the local circumstances of each TB hotspot, and is likely to evolve during our collective progress towards TB control in livestock and in wildlife.

INTRODUCTION

The *Mycobacterium tuberculosis* complex (MTC) is a group of multi-host pathogens thriving at the wildlife–livestock interface. Animal tuberculosis (TB) is due to infection with *Mycobacterium bovis* and other closely related members of the MTC, such as *Mycobacterium caprae*. Zoonotic TB is the disease caused by non-*Mycobacterium tuberculosis* members of the MTC transmitted from animals to humans. It often causes extra-pulmonary disease in humans and is still a major public health concern in developing countries, often linked with the consumption of raw dairy products or close contact with infected livestock (Dürr et al. 2013). In industrialized countries, the main reason for TB control is economic: cattle TB results in severe losses due to trade restrictions and slaughter compensations for test-positive animals (Amanfu 2006). Recently, TB has become of concern in other livestock sectors such as the pig industry (Bailey et al. 2013). TB acquired by

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consuming MTC-infected prey affects lion *Panthera leo* conservation in South Africa (Renwick et al. 2007) and Iberian lynx *Lynx pardinus* conservation in the Iberian Peninsula (López et al. 2014). Moreover, TB-mediated conflicts between farmers, hunters and conservationists can affect animal health and conservation strategies in and around protected natural areas (Gortázar et al. 2010). Hence, animal TB control is considered globally important for public health, economics and conservation.

In most industrialized countries, successful cattle TB control is based on intensive cattle test and slaughter, and on movement control policies, with sporadic whole herd culling (Brooks-Pollock et al. 2014). However, the eradication of cattle TB remains unlikely if the role of all hosts is not clear enough for relevant reservoirs to be targeted at the same time (O'Reilly & Daborn 1995, Gortázar & Cowan 2013). TB control in wildlife is extremely difficult (Fitzgerald & Kaneene 2013). Several reviews have addressed wildlife TB, underlining the need to understand epidemiological complexity and to use integrated approaches for TB control at the wildlife–livestock interface (Gortázar et al. 2012, Palmer et al. 2012, Fitzgerald & Kaneene 2013, Palmer 2013). In this non-systematic review, we describe the significance of wildlife as part of complex multi-host MTC maintenance communities; identify insufficiently known aspects of host pathology, ecology and TB epidemiology; present selected time series in wildlife TB; and summarize ongoing research on TB control. Specific research needs are identified and discussed.

MTC RESERVOIRS

TB control is difficult in large areas with insufficient livestock movement control and complex host communities (Acevedo et al. 2013). MTC host communities are often composed of both domestic and wild hosts. The domestic portion of this host community often includes goats, pigs or other domesticated species in addition to cattle (Gortázar et al. 2011b), while wild hosts often go beyond the local 'key' reservoir species to include additional hosts with larger or smaller contributions to MTC maintenance (Palmer 2013). In addition, the environment itself might contribute to maintaining viable MTC in water or soil, further complicating TB epidemiology (Ghodbane et al. 2014, Walter et al. 2014). More complex scenarios provide better opportunities for MTC survival, and greater challenges for TB control. In this context, the term reservoir means the whole system (domestic hosts, wild hosts and environment) rather than a single-host species (*sensu* Haydon et al. 2002). In our view, cattle are an active part of the MTC maintenance community, while also being the main target of disease control.

Worldwide, the best-known wildlife TB reservoir situations occur in the British Isles and the Iberian Peninsula in Europe (Gortázar et al. 2012), in sub-Saharan Africa (Michel et al. 2006), in parts of North America (Wobeser 2009, Carstensen & DonCarlos 2011, O'Brien et al. 2011a, Shury & Bergeson 2011, Miller & Sweeney 2013) and in New Zealand (Hutchings et al. 2013). These areas represent global wildlife TB hotspots. Details on host ecology, pathology, surveillance and control attempts have recently been reviewed for each of these hotspots by Fitzgerald and Kaneene (2013). Pathology and epidemiology were also recently reviewed by Palmer (2013). Three hotspots are currently regarded as two-host settings, with one target species (cattle) and one single wildlife reservoir: the Eurasian badger *Meles meles* in the British Isles; the introduced Australian brushtail possum *Trichosurus vulpecula* in New Zealand; and white-tailed deer *Odocoileus virginianus* in Michigan, USA. However, the epidemiological complexity might actually be larger in some of these systems.

By contrast, multi-host situations are known to occur in Mediterranean habitats of the Iberian Peninsula and in sub-Saharan Africa. In the Iberian Peninsula, several wild ungulates, namely the Eurasian wild boar *Sus scrofa*, red deer *Cervus elaphus* and fallow deer *Dama dama*, are regarded as members of the wildlife MTC maintenance host community, and TB epidemiology is further complicated by the co-existence of several suitable domestic hosts, including cattle, goats and pigs (Gortázar et al. 2011b). In southern Africa, MTC is probably mainly maintained by the African buffalo *Syncerus caffer* in several settings, and by the lechwe antelope *Kobus leche* in Zambia's Kafue National Park (Michel et al. 2006). But MTC infects a long list of other wild African mammals, some of which might contribute to complexity (Michel et al. 2006).

In addition, MTC infection is endemic in feral pigs on the island of Molokai in Hawaii (Miller & Sweeney 2013), in American bison *Bison bison* in Wood Buffalo National Park, Alberta, Canada (Joly & Messier 2004, Wobeser 2009), and in red deer in the Tyrolean Alps of Austria and neighbouring countries (Schoepf et al. 2012). Figure 1 represents the geographical distribution of scientific literature on wildlife TB, evidencing the existing bias towards a few regions. By contrast, information on wildlife TB from Asia, South America and northern Africa is extremely scarce. Table 1 lists examples of underreported wildlife TB.

Case study: Southeast Asia

One example of a region where the epidemiology of animal TB and the role of wildlife hosts is poorly understood is Southeast Asia. Between 2005 and 2013, the Office International des Epizooties (OIE) World Animal Health Information Database (Anonymous 2013a) recorded TB reports in four countries of the region: Malaysia (75 cases in goats and cattle), Thailand (36 cases in cattle and buffaloes), Myanmar

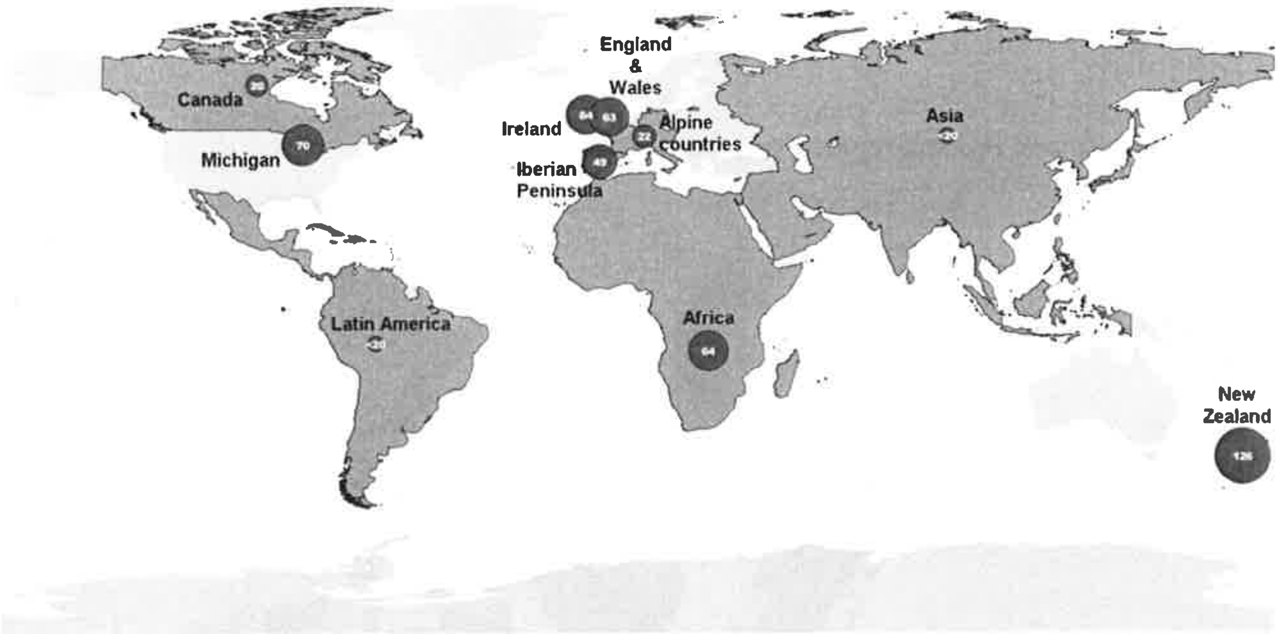

Fig. 1. Numbers of literature references in the Scopus database relating to different geographical areas found using the search terms 'wildlife' and 'tuberculosis' from the period 1994 to 2013. It is evident that scientific activity is biased towards a few hotspots in Europe, North America, sub-Saharan Africa and New Zealand, while very little information is available for Asia and Latin America. Light grey areas are those in which almost no studies of bovine TB were found.

Table 1. Examples of situations where the role of wildlife in *Mycobacterium tuberculosis* complex (MTC) maintenance is unknown, under-reported or still under debate

Host species	Country or region	MTC prevalence and remarks on host status	References
White-tailed deer	Mexico	Low seroprevalence, sporadic confirmation by PCR, reservoir status unknown	Barrios-Garcia et al. (2012); Medrano et al. (2012)
Red deer	Alpine countries, central Europe	Locally high *Mycobacterium caprae* prevalence in red deer, linked to winter feeding, with spill-over to cattle	Schoepf et al. (2012)
Feral pig or introduced wild boar	South America	Sporadic reports on *Mycobacterium bovis* isolation, reservoir status unknown	Meikle et al. (2011)
Eurasian wild boar	Atlantic Spain, France, other regions in Europe	Generally low prevalence, links with cattle TB breakdowns, reservoir status unknown	Muñoz-Mendoza et al. (2013); Richomme et al. (2013)
European bison	Poland	Confirmed infection with spill-over to Eurasian lynx *Lynx lynx* and wild boar, reservoir status unknown	Krajewska et al. (2014)
Eurasian Badger	Atlantic Spain, France, other regions in Europe	Links with cattle TB breakdowns, reservoir status unknown except for the British Isles	Balseiro et al. (2011); Richomme et al. (2013); Payne et al. (2013)
Lion	Kruger National Park, South Africa	Confirmed infection, reservoir status under debate	Renwick et al. (2007)
Deer species and wild boar	England	Confirmed infection, not regarded as reservoirs, but expanding	Ward et al. (2009); Foyle et al. (2010); Ward and Smith (2012)

PCR, Polymerase Chain Reaction; TB, tuberculosis.

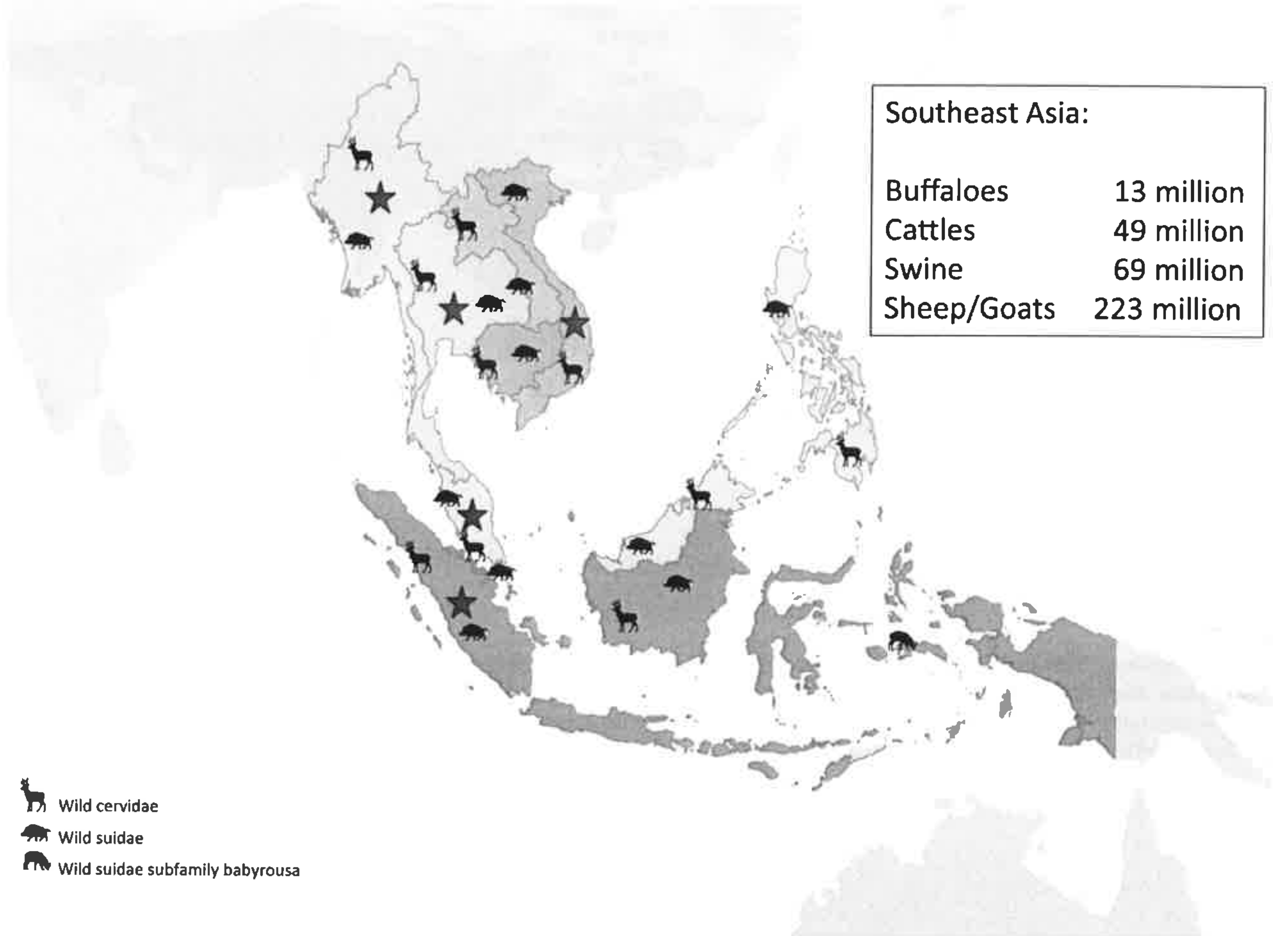

Fig. 2. Map of Southeast Asia, with animal shapes indicating the distribution of potential wildlife hosts for the *Mycobacterium tuberculosis* complex, and stars indicating countries in which animal tuberculosis (TB) was reported between 2005 and 2013 (Myanmar, Thailand, Vietnam, Malaysia and Indonesia). The box shows total estimated livestock numbers (source: Anonymous 2013b).

(9 cases in unknown wildlife species) and suspected cases in wildlife in Vietnam (Fig. 2).

In addition to these OIE data, a literature review revealed the possible implication of other animal species in MTC epidemiology, including Asian elephants *Elephas maximus* (Angkawanish et al. 2010, but see Ong et al. 2013) and non-human primates (Payne et al. 2011). Hence, TB is present in the region both in livestock and in wildlife.

However, the extent of the problem and the role of wildlife in MTC epidemiology are largely unknown. Wild suids *Sus scrofa* for instance are regarded as suitable MTC maintenance hosts in some parts of Europe (Gortázar et al. 2012) and America (Miller & Sweeney 2013). In Southeast Asia, wild suids (several species) are widely distributed (Fig. 2), and occur at high densities (e.g. up to 78 per square km in Malaysia, Ickes 2001). Similarly, deer of the subfamily cervinae (genera *Axis* and *Cervus*) are present in Southeast Asia (Fig. 2). Thus, after improving the knowledge on TB in livestock, it would be interesting to investigate the presence and prevalence of TB in wild suids and deer from different sites throughout the region, including sites with and without contact with livestock, and representing the range of wild ungulate densities. Since contact between domestic and wild suids is likely in Southeast Asia, there is an increasing trend in deer farming, and trans-border movement of potential wildlife reservoirs is likely, wildlife hosts need to be studied along with the domestic MTC hosts.

Research needs: MTC reservoirs

Two aspects require further research: first, completing the map of wildlife MTC reservoirs (as noted by Palmer 2013) and second, understanding the structure of each regional MTC host community: host species and their roles.

HOST PATHOLOGY, ECOLOGY AND TB EPIDEMIOLOGY

The distribution and characteristics of TB lesions in infected hosts of different species is of paramount importance for understanding MTC transmission. It is assumed that TB lesion characteristics and their distribution are related to mycobacterial excretion and may be related to transmission risk. In ungulates (bovids, cervids and suids), lung and thoracic lymph node involvement is frequent (about 20–60% of infected individuals; O'Brien et al. 2001, Martín-Hernando et al. 2007, 2010, Shury & Bergeson 2011, Fitzgerald & Kaneene 2013, Muñoz-Mendoza et al. 2013). In white-tailed deer, the most common site for lesion development is the medial retropharyngeal lymph node (Palmer 2013). This is consistent with MTC shedding mainly by oral and nasal excretion and secondarily also by faecal excretion. In badgers, the prevalence of lung involvement is also about 50%. However, kidneys are often affected, too, and infected bite-wounds or draining subcutaneous lymph nodes might also produce additional shedding routes (Gallagher & Clifton-Hadley 2000). Badgers are thought to transmit MTC to cattle mainly by indirect contact, for instance by contaminating cattle feed (Wilson et al. 2011). Infected possums rarely present lung lesions, but do develop draining lesions in subcutaneous inguinal and axillary lymph nodes, which are likely to contribute to inter-species transmission (Paterson & Morris 1995). These different potential MTC shedding routes might have significant consequences for TB epidemiology, including environmental contamination. Knowing the shedding routes may contribute to improved control.

Host sex and age are well-known predictors of risk of MTC infection. Other less often evidenced individual risk factors include belonging to infected family groups or social groups (e.g. Blanchong et al. 2007, Gortázar et al. 2011a), and certain individual genetic backgrounds (Acevedo-Whitehouse et al. 2005). Known risk factors at the population level include having direct and indirect contact networks (risk varies with inter-specific contact rate) to other infected host species, existing at high densities and forming large spatial aggregations at feeding sites or waterholes (O'Brien et al. 2006, Vicente et al. 2007b). Co-infections have been suggested to drive TB epidemiology. In Mediterranean Spain, correlations were found (at the population level) between wild boar TB prevalence, generalized TB and the prevalence of porcine circovirus type 2, a widespread immunosuppressive virus (Risco et al. 2013). However, such correlations were less evident at the individual animal scale (Díez-Delgado et al. 2014b). Human influences (via activities such as fencing, feeding, translocating) have often contributed to the introduction and maintenance of TB in wildlife (Palmer 2007, Fitzgerald & Kaneene 2013, Vicente et al. 2013). Thus, while co-infections could be relevant, confounders such as management impede our understanding of them in non-experimental settings.

Not all infected individuals contribute equally to MTC maintenance within a certain host category and in a given epidemiological setting. Animals with generalized lesions are more likely to shed MTC (Palmer 2013 and the authors, unpublished data), and those that do not shed may play a limited epidemiological role or not play a role at all (Corner 2006). Unfortunately, the drivers of TB generalization are less well-known than the drivers of infection, since lesion scoring is not carried out as frequently as is the simple detection of infection. In fact, proper quantitative or semi-quantitative lesion scoring is usually only done in experimental infections (e.g. Ballesteros et al. 2009b), as opposed to observational studies in the wild (Vicente et al. 2013). For instance, the practical necessity of maintaining large-scale surveillance programmes over long periods often restricts routine sampling to those anatomical sites (e.g. head region) that are most frequently infected.

It is interesting to note that, while in TB-endemic Mediterranean regions, 57% of MTC-infected wild boar develop thoracic lesions (Martín-Hernando et al. 2007), less than 20% do so in non-endemic areas of Atlantic climate such as Asturias in northern Spain (Muñoz-Mendoza et al. 2013). This suggests that in non-endemic areas, some factors, maybe lower infection pressure, less frequent re-infection events, or differences in the rate of co-infections and in general host condition, contribute to limiting the likelihood of animals developing severe disease. In Spain, dry seasons have been identified as one of those drivers of TB generalization in wild boar (Vicente et al. 2013).

TB is largely regarded as a respiratory disease, in which airborne transmission by aerosols plays a major epidemiological role (Hopewell 1994). However, there is increasing scientific evidence suggesting that direct contacts (those facilitating aerosol transmission) between species are scarce (Kukielka et al. 2013), and direct intra-species transmission is not easily replicated in experiments (e.g. lack of transmission among fenced feral pigs in New Zealand, Nugent 2011). Moreover, observational (Vicente et al. 2007b) and experimental studies (Palmer et al. 2004, Barasona et al. 2013) have shown that indirect transmission can occur at feeding sites or waterholes. Considering this information together, we hypothesize that MTC transmission between wild and domestic hosts is mostly indirect.

Research needs: host pathology, ecology and TB epidemiology

Research should focus on delivering better knowledge of the origin and behaviour of generalized tuberculous individuals within populations, and of the role of co-infections, re-infections and individual condition (among other factors) in TB pathogenesis. Research quantifying indirect MTC transmission within and between species is duly needed to inform decisions on investment in control options that tackle different potential transmission routes.

TIME TRENDS IN WILDLIFE TB

Unfortunately, few long-term studies in which serial monitoring is conducted are available for wildlife TB. A selection of the longer ones is shown in Fig. 3. Generally speaking, stable or declining trends below 10% prevalence occur in cervids (e.g. elk *Cervus elaphus* in Riding Mountain National Park, Canada) and in badgers in Woodchester Park, England, while increasing trends occur in African buffalo in the Kruger National Park, South Africa and in wild boar in Ciudad Real, Spain. The available information represents changes over time in prevalence, as opposed to true time trends. Comparisons between species are difficult due to variability in data type.

Analysing the significance of these trends and inferring their drivers is beyond the scope of this review. However, one could argue that wildlife TB control has been relatively successful in (almost single-host) North American cervid populations, as shown by the progressive declining trend of white-tailed deer TB in Michigan, and the success of interventions in Minnesota (Carstensen & DonCarlos 2011) and Manitoba (Shury & Bergeson 2011); while in high-prevalence populations in multi-host settings with limited (if any) control intervention, TB keeps getting worse (e.g. Vicente et al. 2013). We hypothesize that two factors determine the observed trends: the capability to intervene with costly or potentially contentious measures such as population control, feeding bans and biosafety improvements; and intervention in single-host vs. multi-host settings.

Research needs: time trends

There is a clear lack of constant, harmonized information derived from large-scale disease and population monitoring. Even the best available time series data refer to local areas, and information on wildlife abundance and relevant metadata is not as readily available as information on TB prevalence in livestock or even in wildlife. Hence, there is an urgent need to define and harmonize wildlife disease monitoring protocols, and to apply them in a way that allows proper trend comparisons in both space and time. This information is of paramount importance for assessing the effect of any future intervention (Boadella et al. 2011, Gortázar et al. 2015).

ATTEMPTS TO CONTROL TB

The goal of cattle TB eradication requires the development of strategies that reduce pathogen transmission between wildlife and domestic animals (O'Reilly & Daborn 1995). This is a cross-disciplinary task where inter-agency teams of vets and ecologists are needed (Fitzgerald & Kaneene 2013). Currently available tools for disease control at the wildlife–livestock interface were recently reviewed (Delahay et al. 2009, O'Brien et al. 2011b, Gortázar et al. 2015) and range from interaction management (farm biosafety improvement), through random (culling) or targeted (test and cull) population control, to vaccination. Examples of successful MTC eradication in wildlife are scarce, although there are cases consistent with wildlife culling impacting on cattle TB (see Table 2). One aspect to consider is reporting bias: failed experiments are less likely to be reported than successful ones.

Population control

The UK randomized badger culling trial (Donnelly et al. 2006, 2007), and the Irish four areas experiments (Corner et al. 2008), along with data on wild boar culling in Spain (Boadella et al. 2012), are among the few studies where random culling was tested as a single TB control tool. In our view, random culling of wildlife reservoir hosts might contribute to cattle TB control through reduction of densities,

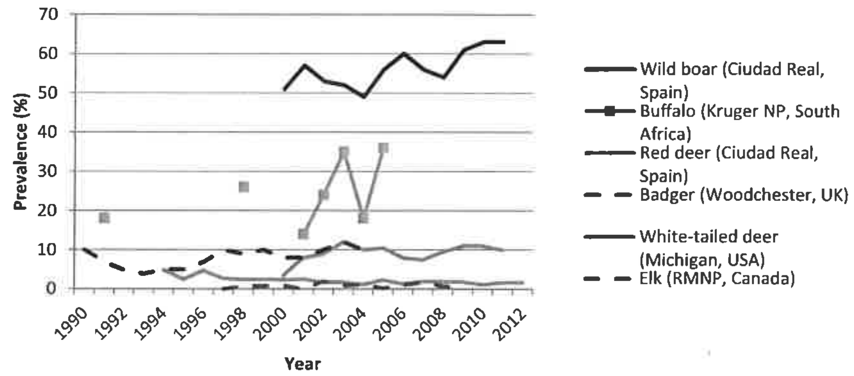

Fig. 3. Selected time trends of tuberculosis (TB) prevalence in wildlife hosts. Source: authors' estimates based on figures in Cross et al. 2009, O'Brien D.J. (personal communications), Shury and Bergeson 2011, Vicente et al. 2007a, 2013. The UK badger data refer to prevalence of animals found to be excreting bacilli, not the prevalence of infected animals.

Table 2. Selected examples of tuberculosis (TB) control attempts at the wildlife–livestock interface and their reported outcomes

Host and setting	TB control tools	Outcome (in wildlife and cattle)	References
Wild boar in multi-host settings, Spain	Wild boar population control, (also in cattle depopulation and re-stocking)	Variable outcomes, including reduction in wild boar TB prevalence, and in TB prevalence in sympatric deer and cattle	Boadella et al. (2012); Garcia-Jimenez et al. (2013); Mentaberre et al. (2014)
Badger in the British Isles, UK and RoI	Badger population control	Variable, depending on study site, treatment surface and type of control. Positive effects on cattle TB in Ireland.	Corner et al. (2011)
White-tailed deer in Minnesota, USA	White-tailed deer population control, feeding bans, cattle biosafety improvements, voluntary cattle buyout programme	TB no longer detectable in wildlife, cattle herds cleared	Carstensen and DonCarlos (2011)
White-tailed deer in Michigan, USA	White-tailed deer population control, feeding bans, cattle biosafety	Significant reduction, but TB still endemic in deer, sporadic in cattle, other wildlife	O'Brien et al. (2011a); Ramsey et al. (2014b)
Buffalo and other mammals in Kruger NP, South Africa	Electrified fencing to avoid escapes and contact with cattle	Some species are able to cross fences	Renwick et al. (2007)
Wild boar and red deer on beef cattle farm, Spain	Changes to waterholes making them cattle or wildlife-proof	Reduction in cattle TB prevalence below previous records and below control areas	Barasona et al. (2013)
Brushtail possum, New Zealand	Oral BCG vaccination	Lower incidence in vaccinated possums	Tompkins et al. (2009)
Badger, UK	Parenteral BCG vaccination	Lower test positivity in vaccinated badgers, unvaccinated cubs. Lower infection likelihood in vaccinated sets	Chambers et al. (2011); Wilson et al. (2011); Carter et al. (2012)
Wild boar, Spain	Oral IV vaccination	Lower infection prevalence in vaccinated wild boar	Díez-Delgado et al. (2014a)
Brushtail possum, New Zealand	Nationwide population control (poison)	Lower possum TB prevalence and less spill-back to cattle. Cattle TB declining.	Hutchings et al. (2011)
White-tailed deer, Michigan, USA	Local test and cull attempt	Impractical due to low prevalence; expensive, eradication unlikely	Cosgrove et al. (2012a, b)
American bison, Elk Island National Park, Canada	Local test cull attempt	Success (small isolated population)	Wobeser (2009)

BCG, Bacille Calmette-Guérin; RoI, Republic of Ireland.

contact rates or specific high-risk individuals. However, it provokes a strong debate among stakeholders and is not sustainable as a long-term tool in Europe. Possible exceptions might include culling invasive (pest) species such as possums in New Zealand (O'Brien et al. 2011b) or water buffalo *Bubalus bubalis* in Australia (Radunz 2006), and short-term culling in response to new outbreaks (Carstensen & DonCarlos 2011, Gortázar et al. 2015).

Targeted culling (selective culling) failed to yield convincing results in attempts targeting white-tailed deer in Michigan (Cosgrove et al. 2012b) or wild boar in Spain (the authors, unpublished data). This tool depends on capturing a large proportion of an animal population, testing it, and selectively removing test-positive individuals. This kind of intervention is probably more suitable for captive wild animals (Gortázar et al. 2015).

Farm biosafety

Farm biosecurity practices are known preventative tools in livestock farming. The rationale consists of using fencing and other physical barriers to reduce contact between livestock and potentially infected wildlife (Gortázar et al. 2015). For instance, sheet metal gates and fencing, feed bins and electric fencing were shown to prevent badgers from entering cattle farm facilities in the UK (Judge et al. 2011). In Spain, segregating wild ungulates and livestock from common resources such as waterholes reduced cattle TB incidence (Barasona et al. 2013). Nonetheless, fences are vulnerable to certain animal species that can destroy them, and are expensive to set up and maintain (Jori et al. 2011). As in the case of culling, there are few situations where biosafety improvements have been the only approach to TB control at the wildlife–livestock interface (Renwick et al. 2007, Barasona et al. 2013).

Research needs: controlling TB

There is a need for experiments enabling the evaluation of the benefits of each single intervention tool. Although valuable examples of on-farm prevention do exist, there is no replicated information available on the effect on cattle TB of using preventive farm biosafety measures alone, in the absence of other interventions such as feeding bans or population control.

VACCINATION

Of the three main intervention tools, namely biosafety measures, culling and vaccination, vaccination is most likely to provide hope in the decade ahead. This is because biosafety measures alone can help to reduce cattle TB prevalence, but are unlikely to eradicate MTC (Barasona et al. 2013), and because culling is generally regarded as a non-sustainable disease control tool in the long term (Chambers et al. 2011, Corner et al. 2011, Boadella et al. 2012).

Recent reviews have addressed wildlife TB vaccination (O'Brien et al. 2011b, Beltrán-Beck et al. 2012, Buddle et al. 2013, Gormley & Corner 2013). Controlled and replicated field experiments are available for possums (Tompkins et al. 2009), badgers (Chambers et al. 2011) and for wild boar (Díez-Delgado et al. 2014a). Here we use two case studies to discuss some practical aspects of wildlife vaccination in single-host and multi-host contexts.

Case study: planning for vaccination in Michigan, USA

Vaccination of wildlife to eliminate pathogens at landscape scales has proven nearly impossible where multiple maintenance hosts exist (Plumb et al. 2007). Because only one maintenance host other than cattle, i.e. white-tailed deer, has thus far been identified in Michigan (O'Brien et al. 2006, 2011a), vaccination is a viable option, and has been included in modelling of likely outcomes of control strategies (Ramsey et al. 2014a).

In Michigan livestock, 67 TB-positive herds have been identified (49 beef, 13 dairy cattle, 4 farmed deer and 1 bison). Forty-five outbreaks (67%) have been officially attributed to wildlife exposure. Eight cattle farms have experienced multiple breakdowns (7 twice, 1 three times), accounting for 27% (17 out of 62) of the cattle farm total. Elimination of TB from Michigan cattle appears to be achievable: the number of herds that break down each year is low (mean: 3.7, range: 1–8, variance: 2.2 in 1998–2014), most herds had two or fewer culture-positive cattle at post mortem, electronic identification has theoretically made all Michigan cattle traceable, and improvements in farm biosecurity have been partially implemented (O'Brien et al. 2011a, Walter et al. 2012). Who would pay for vaccination has not yet been seriously discussed.

One argument justifying vaccination of white-tailed deer is stewardship, the responsibility to leave a healthy deer herd for sustained use by future generations. Notably, vaccination is not justified by negative population-level effects of bovine TB on white-tailed deer. White-tailed deer are abundant: the estimated population in Michigan is 1.7 million (range 1.69–1.97 million, 2011–2013; Michigan Department of Natural Resources, unpublished data; estimation via sex–age kill; Mattson & Moritz 2008). Population-level TB mortality is minimal, and white-tailed deer are of economic and cultural, rather than conservation, significance (O'Brien et al. 2006). Vaccinating deer is arguably less justifiable than vaccinating cattle, but vaccination of white-tailed deer is partially driven by the current lack of a viable bovine vaccine, and by social concerns that make cattle an unpalatable target for vaccination (Plumb et al. 2007). Cattle vaccination is likely to be far simpler logistically and more cost-effective than deer vaccination. Ultimately, however, vaccination of white-tailed deer is justified to eliminate the self-sustaining wildlife reservoir from the landscape.

Prevention of TB in white-tailed deer, while desirable, is not necessary. Vaccination needs only to reduce or prevent transmission among deer and to other species. As the vaccine, Danish Bacille Calmette-Guérin (BCG) is the likely candidate (Waters et al. 2012). In 19- to 20-week studies, BCG has proven safe and effective (Palmer et al. 2007, 2009, 2014b, Nol et al. 2008). Oral administration has been most effective (Nol et al. 2008). Given the greater tissue persistence of parenteral BCG (Palmer et al. 2010b, 2014c), oral administration seems likely to be the preferred delivery route. Research into bait uptake is underway (Palmer et al. 2014a). Limited secondary transmission of BCG has occurred from vaccinates to unvaccinated in-contact deer, but not to indirectly exposed cattle (Palmer et al. 2010a, Nol et al. 2013). Studies of duration of immunity, reduction of transmission, and the effects of BCG overdose in white-tailed deer are underway (M. V. Palmer, personal communications). The geographical scale of a potential vaccination programme is relatively well-defined: both white-tailed deer and cattle in the core outbreak area, Deer Management Unit (DMU) 452, have the highest risk of infection based on prevalence (Table 3). Within DMU 452, cattle farms occupy less than one-third of the area (Fig. 4). Modelling suggests that vaccinating deer in the vicinity of those farms could quickly reduce herd breakdowns (Ramsey et al. 2014b).

Female white-tailed deer and their female offspring exhibit remarkable fidelity to natal range (Van Deelen et al. 1998, Nelson & Mech 1999). In the TB-endemic area, most

Table 3. Apparent prevalence of *Mycobacterium bovis* infection in free-ranging white-tailed deer and percentage of cattle farms with tuberculosis (TB+) by geographical area, Michigan, USA, 1975–2014

Area	TB+ free-ranging white-tailed deer		TB+ cattle farms	
DMU 452	579/27476	2.1%	16/88	18%
Rest of endemic area	143/53010	0.27%	24/585	4.1%
Rest of Michigan	22/120759	0.018%	13/~12953*	0.1%

DMU, Deer Management Unit.
*(Anonymous 2014).

females first breed as yearlings; about half produce twins. Females typically give birth to twins annually from age 2 onward. If infected, matriarchial groups are likely to maintain bovine TB locally, and males act as between-group spreaders (O'Brien et al. 2002, Palmer et al. in press). Annual mean white-tailed deer harvest in DMU 452 is ~5400 of the population of ~25000–30000, comprising 40% of the adult male and 16% of the female and fawn population per year. Between 2001 and 2011, 40% (range 31–49%) of the white-tailed deer harvested were ≤1.5 years old (Michigan Department of Natural Resources, unpublished data). If, as in other species (Ballesteros et al. 2009a, Waters et al. 2012), young white-tailed deer are optimal targets for vaccination, increased hunting pressure on juveniles resulting in high juvenile mortality may prolong the time to bovine TB eradication. Annual vaccination of 90% of susceptible deer with a 90% efficacious vaccine would take about three decades to achieve eradication (Ramsey et al. 2014a). Models predict that the shortest time to eradication will occur by vaccinating deer in midsummer (Palmer et al. in press). However, the desire to minimize human exposure to persistent BCG in harvested venison (Palmer et al. 2010b, 2014c) may dictate vaccination in winter.

Designing a field trial presents many challenges. A combined field feasibility and modelling study of a live-trap/test/cull or vaccinate approach suggested that 30 years of application in DMU 452 would cost ~US$50 million and carry only a 34% probability of bovine TB eradication from white-tailed deer (Cosgrove et al. 2012a, b). Consequently, mass distribution of oral baits is likely to be necessary. Costs notwithstanding, sacrificing large numbers of deer in order to measure the effectiveness of vaccination may not be feasible because of opposition by hunters. Potential alternatives may include greatly expanded testing of hunter-harvested deer for BCG, and a before–after control–impact study to measure the rate of TB extinction (MacKenzie et al. 2006). Delivery of oral BCG could occur either via bait stations (Ballesteros et al. 2009a) or via aerial bait drops (Rosatte et al. 2009, Muller et al. 2012). Accomplishing uniform spatial coverage with bait stations would necessitate unfettered access to privately owned lands, which comprise 93% of DMU 452 (Carstensen et al. 2011). Increased aggregation of deer at bait stations (Thompson et al. 2008) and the consequent increased TB transmission (Becker & Hall 2014, Ramsey et al. 2014a) might attenuate vaccination's positive effects. Agency use of bait where baiting by hunters is banned could prove problematic (Rudolph et al. 2006).

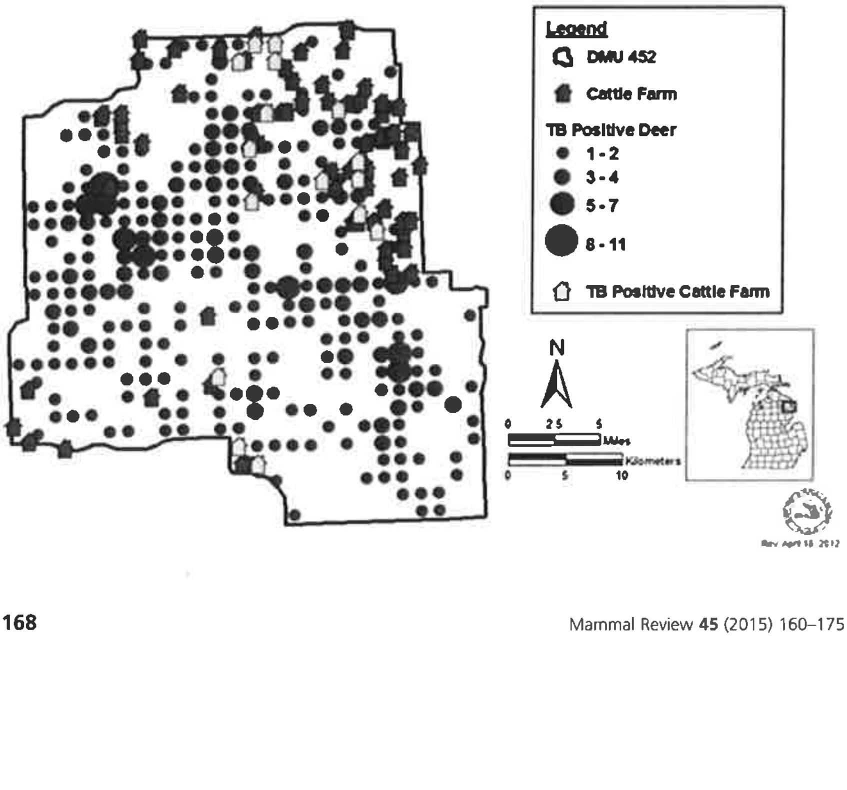

Fig. 4. Locations of *Mycobacterium bovis* infected free-ranging white-tailed deer and tuberculosis (TB)-positive cattle farms in Deer Management Unit 452, Michigan, USA, 1975–2012.

Aerial bait distribution would be likely to lead to better spatial coverage of white-tailed deer, but there would be less control of BCG exposures to non-target species, notably cattle.

Given current modelling results, it seems that no strategy, including vaccination, will be successful with only short-term commitment to its application. It is not yet clear whether Michigan's policymakers, hunters, farmers and public are sufficiently committed to TB eradication to warrant embarking on a long-term vaccination programme in earnest (Ramsey et al. 2014a).

Case study: Montes de Toledo, Spain

Starting in 2007, the first controlled laboratory wild boar vaccination trials employed oral and parenteral BCG and resulted in the characterization of the wild boar immune response to vaccination and infection (Ballesteros et al. 2009b, Pérez de la Lastra et al. 2009). Later, a heat-inactivated field strain of *Mycobacterium bovis* (inactivated vaccine, IV) was successfully used in new controlled vaccination and challenge trials. The IV yielded protection levels similar to BCG (Garrido et al. 2011). In additional experiments in captive animals, protection levels of above 80% were achieved through re-vaccination, both for BCG (Gortázar et al. 2014) and for IV (Beltrán-Beck et al. 2014a). In parallel, suitable oral baits (Ballesteros et al. 2009c), piglet-selective deployment cages (Ballesteros et al. 2009a) and efficient bait deployment strategies were designed and field-tested (Ballesteros et al. 2011). Since the summer of 2012, the first field trials are ongoing in the high-prevalence region of Montes de Toledo in Spain using BCG and IV in different sites (Díez-Delgado et al., unpublished data).

Safe and specific vaccine deployment is a key concern in oral vaccination strategies. In addition, confirmation of bait uptake (i.e. the use of marked baits) is needed to generate sound scientific data. In the wild boar field vaccination experiments, safety and specificity were confirmed through camera trap surveys and analyses of target and non-target host tissues for BCG (Beltrán-Beck et al. 2014b). Furthermore, daily bait deployment at dusk and collection of non-consumed baits immediately after dawn improved bait specificity and limited BCG inactivation due to high environmental temperatures. However, this procedure is labour-intensive and could be avoided by deploying only the IV.

Assessing vaccination efficacy under field conditions is challenging. In the ongoing vaccination field trials, sites were purposefully selected away from cattle farms, since risks of cattle contamination with live BCG could not be excluded *a priori*. Hence, no results will become available in terms of reductions in outbreaks of cattle TB. So far, this has also been the case in other field vaccination trials in possums and badgers (Tompkins et al. 2009, Chambers et al. 2011). Further experiments on and around TB-positive cattle farms are required. However, the effects of wild boar vaccination are measured in the wild boar target host. Efficacy can be tested at the individual level (in piglets with and without the biomarker) and at the population scale (treatment sites before and after treatment; treatment sites vs. controls). Preliminary information regarding lesion and culture scores in piglets is encouraging (Díez-Delgado et al., unpublished data).

No cost estimations are currently available for this field experiment. Also, results are currently available for only two bait deployment years, and the experiment will continue for at least 4 years. Modelling suggests that clear results at the population scale will become evident after 5 years of vaccination (Anderson et al. 2013). Finally, the newly developed vaccine and selective baiting tools require knowledge transfer from the laboratory to the market, and attainment of regulatory approval for distribution in the field. Here, the challenges faced are beyond those of traditional science, but are equally important.

Research needs: vaccination

As shown by the case studies, field vaccination trials can be challenging in terms of deployment logistics and accurate efficacy assessment, and often need to take place over periods longer than those covered by the usual research grant funds. Some critical aspects to be considered are not scientific, but include cost-efficiency analyses and knowledge transfer aspects.

DISCUSSION

In a review on wildlife disease monitoring using mycobacterial diseases as a case study, Boadella et al. (2011) identified several conceptual steps, from disease discovery and descriptive epidemiology, through risk factor identification and monitoring, to disease control. In this review, we identify six specific research needs, broadly corresponding to these conceptual steps, as follows:

1. Complete the world map of wildlife MTC reservoirs and describe the structure of each local MTC host community, and the role of the environment (disease discovery and descriptive epidemiology).

2. Identify the origin and behaviour of generalized diseased individuals within populations, and study the role of factors such as co-infections, re-infections and individual condition on TB pathogenesis (risk factor identification).

3. Quantify indirect MTC transmission within and between species (risk factor identification).

4. Define and harmonize wildlife disease monitoring protocols, and apply them in a way that allows proper population and prevalence trend comparisons in both space and time (monitoring).

5. Carry out properly designed wildlife TB control experiments enabling the evaluation of the benefits of each single intervention tool.

6. Analyse cost-efficiency and consider knowledge transfer aspects in promising intervention strategies (control).

We believe that addressing these points would push forward our capacity for TB control, through improving our ability to identify, measure and control TB in multi-host systems. However, many other open questions remain. Vaccination, for instance, usually targets a single-host species. Where MTC is maintained in a host community, the benefits of targeting just one host species might be limited (Plumb et al. 2007).

Another question derives from the fact that human influences have often contributed to the introduction and maintenance of TB in wildlife (Carstensen et al. 2011, Fitzgerald & Kaneene 2013, Vicente et al. 2013). Efforts to deal with social aspects and management-related public conflicts deserve more support in order to achieve better stakeholder and society buy-in (O'Brien et al. 2011a). For instance, farmers' opinions about TB can be influenced by their experience of the disease and their interactions with wildlife (e.g. hunters vs. non-hunters; Cowie et al. 2013).

Since TB is more difficult to control in wildlife than in cattle (Fitzgerald & Kaneene 2013), and since interventions in natural systems are prone to conflicts (Artois et al. 2001), a further remaining question is whether or not interventions on wildlife TB are at all justified. The answer varies depending on the local circumstances in each TB hotspot, and is likely to evolve during our collective progress towards TB control in livestock and in wildlife.

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