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BCG VACCINATION FOR BOVINE TUBERCULOSIS; CONCLUSIONS FROM THE JERSUSALEM ONE HEALTH WORKSHOP

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Abstract

The global burden of bovine tuberculosis (bTB) remains poorly characterized, with spill-over impacts on multiple species. The “One Health” concept is especially relevant given the bidirectional risk of cattle infecting humans with *Mycobacterium bovis* and humans infecting cattle with *M. tuberculosis*. “Test and cull” is the traditional bTB control method, but the strategy may not be economically feasible or culturally acceptable where cattle are highly prized or their killing is a religious taboo; it is also less effective when there are wildlife reservoirs of infection. Vaccination with *M. bovis* bacille Calmette-Guerin (BCG) provides protection against bTB, but its use in animals has been limited.

The Jerusalem One Health workshop considered key bTB knowledge gaps and innovative solutions. Knowledge gaps identified included 1) the poorly quantified prevalence of *M. bovis* infection and disease in cattle, domestic camelids and human populations in developing countries, 2) the absence of alternatives to a “test and cull” strategy in settings where the killing of infected animals is culturally or economically unacceptable, or where affected species are protected, and 3) an understanding of the induction of mucosal immunity against bTB. We summarize discussions on the use of BCG vaccination in domestic animals and wildlife and list potential projects to address the knowledge gaps identified.

Introduction

The German pathologist Rudolph Virchow coined the term “zoonosis” to describe diseases common to humans and animals (Thirunavukkarasu 2017); preceding our appreciation that the majority of infectious diseases affecting humans today have an animal origin (Wolfe, 2007). In recent times growing international trade and travel, as well as increased ecosystem fragmentation and human animal contact, have facilitated the emergence and spread of pathogenic organisms (Rabinowitz 2009, Hill-Cawthorne 2017). The “One Health” concept acknowledges that the health of humans and animals are closely interlinked and emphasizes the fact that all animals (including humans) provide a potential reservoir for pathogenic species (Thirunavukkarasu 2017). Bovine tuberculosis presents a classic One Health challenge, since disease reduction in cattle will improve bovine health, and at the same time will reduce health risks to humans and wildlife.

Bovine tuberculosis (bTB) has proved to be an intractable problem in many countries, particularly in settings where “test and cull” policies are not affordable or socially acceptable, or in areas where *Mycobacterium bovis* infection is sustained by wildlife reservoirs.

Indications are that bTB causes a substantial global disease burden (WHO 2017), but in the absence of reliable surveillance data it is impossible to assess the true incidence of *M. bovis* infection in humans, domestic cattle and camelids, and various wildlife species. Although humans mostly acquire *M. bovis* infection from cattle (Amato 2018, Sandoval-Azuara 2017), indications are that in some parts of the world cattle with presumed bTB may in fact be infected with *M. tuberculosis* and not *M. bovis* (Mittal 2014, Sweetline 2017, Hlokwe 2017); reflecting likely human to cattle transmission of tuberculosis (TB). These examples of “reverse zoonosis” provide a powerful reminder that pathogen transmission between animals and humans is bidirectional. Genomic evidence indicates that human TB caused by *M.*

tuberculosis did not evolve from *M. bovis* as previously thought; in fact, animal strains included in the *M. tuberculosis* complex likely evolved from ancestral human strains, although further elucidation is required (Smith 2009).

Globally, the annual costs to deal with bTB have been estimated at US\$3 billion (WHO 2017). India has the largest population of cattle in the world - around 300 million - of which an estimated 22 million is infected with *M. bovis* (Srinivasan 2018), but bTB control efforts are compromised by inadequate disease surveillance and religious objections to the slaughter of infected animals. As in other parts of the developing world, cattle are mainly kept in small holdings and represent a major source of livelihood, nutrition and cultural wealth. Given the limited efficacy of traditional bTB containment methods in large parts of the world, it seems important to re-assess the value of alternative approaches, especially the potential benefits of bacille Calmette-Guérin (BCG) vaccination. BCG vaccination has been used in humans for nearly 100 years, but its use in animals remains limited, despite the fact that Calmette and Guerin tested BCG in cattle in 1911; 10 years before its use in humans (Waters 2012). With the exception of Great Britain where BCG vaccine has been licensed for use in badgers (made possible by the licensing in 2010 of BCG for intramuscular administration to badgers – BadgerBCG®) (Perrett 2018), widespread use of BCG in animals has not been implemented.

Jerusalem workshop

A bTB workshop held in Jerusalem, Israel, from 28 February to 2 March 2018 aimed to review challenges in bTB control across the One Health spectrum and to evaluate recent progress in BCG development, including innovative mucosal delivery methods. For the purposes of the discussion *M. bovis* was considered the main bTB pathogen, although bTB may be caused by other mycobacteria belonging to the *M. tuberculosis* complex, while *M.*

bovis also infects non-bovine species. The meeting built upon the recently released World Health Organization (WHO) “Roadmap for zoonotic tuberculosis: a One Health approach to ending tuberculosis” (WHO 2017), and a Gates Foundation sponsored bTB meeting held in Rabat, Morocco in 2015 - “Accelerating bTB Control in Developing Countries”. Participant expertise included bTB in domestic cattle, camelids and wild animals, TB caused by *M. tuberculosis* and *M. bovis* in human populations, mycobacterial genomics, mucosal immunity, BCG vaccination and oral vaccine delivery methods, as well as vaccine manufacturing processes. Participants shared their personal experience and research results, while discussions focussed on global bTB control issues.

The group identified key knowledge gaps and research needs in bTB control, and articulated the rationale for specific studies to address these research needs (Table 1). Knowledge gaps identified include 1) the poorly quantified prevalence of *M. bovis* infection and disease in less developed parts of the world, 2) the absence of alternatives to a “test and cull” strategy in settings where the killing of infected cattle or wildlife is culturally or economically unacceptable or impossible given the protected status of certain wildlife species, and 3) the fact that optimal and pragmatic BCG vaccine delivery options remain elusive. Since the need for better bTB surveillance and implementation of traditional control methods in developing countries was strongly emphasized in the recent WHO Roadmap (WHO 2017), our discussions focused mostly on BCG vaccination as an underutilized bTB control strategy.

Efficacy of oral BCG

Although human BCG today is primarily administered through the intradermal route, oral BCG administration has a long history and was used in multiple controlled studies performed in the 1920s and 30s; demonstrating significant protection against TB (Aronson 1935,

Kerszturi 1929). More recent studies showed that oral BCG administration induces mucosal immunity, with enhanced TB-specific secretory IgA, T-cell homing to restricted lung mucosal compartments and bronchoalveolar lavage recovery of these T-cells, compared to intradermal vaccination (Hoft 2018, Lai 2015) . In studies using rhesus macaques, pulmonary mucosal BCG vaccination conferred enhanced protection compared to standard intradermal BCG (Verreck 2017), and it seems preferable to match the route of vaccination and natural infection (Manjaly 2015). In cattle, the route of transmission is primarily by aerosol, although calves are commonly infected through ingestion of infected milk, while for wildlife, transmission can be via a variety of routes involving aerosol inhalation or oral ingestion. Therefore induction of mucosal immunity could be beneficial and oral vaccination with BCG might be advantageous. Preliminary research into the use of oral BCG to protect cattle against bTB has been encouraging,(Buddle 2018; Nugent 2017) as has been its application in wildlife reservoirs, such as possums in New Zealand (Tompkins 2009) and badgers in Ireland (Gormley 2017). This experience, as well as the effective administration of rabies vaccine using bait, suggests that oral administration of BCG vaccine might be the most cost-effective means of vaccinating wildlife (Pastoret 1996).

BCG vaccination of cattle

Studies over the past two decades have demonstrated that BCG vaccination of cattle can be a valuable tool in bTB control, especially in settings where “test and cull” is not an option or persistent wildlife reservoirs are difficult to eradicate (Buddle 2018). Experimental challenge studies have identified many of the variables which influence BCG efficacy in cattle.

Although BCG vaccination does not induce complete protection, no single vaccine has proven to be more efficacious than BCG to protect cattle against bTB. Recent studies have shown that similar levels of protection were induced when BCG was applied parenterally or

orally, although higher doses were required for oral vaccination (Buddle 2018). The highest level of efficacy was achieved when calves were vaccinated at <1 month of age, followed by a revaccination boost at 12-24 months to prevent waning immunity (Parlane 2014). Although assessment of BCG as an effective bTB control strategy is challenging (Conlan 2018), field studies in Ethiopia, Mexico and New Zealand have established that BCG vaccination offers a significant level of protection against natural *M. bovis* infection (Ameni 2010, Lopez-Valencia 2010, Nugent 2017, Nugent 2018).

A major constraint using BCG vaccination in cattle is the fact that trading blocs like the European Union prohibit the use of TB vaccines in cattle, since vaccination compromises the interpretation of bTB diagnostic tests such as the traditional tuberculin skin test (TST). These concerns are currently being addressed with the development of more specific tests that are able to differentiate infected from vaccinated animals (DIVA). These skin tests and whole blood interferon- γ assays utilize *M. bovis* specific antigens that are not expressed in BCG, eliminating concerns that BCG vaccination might compromise bTB diagnosis (Vordermeier 2016). Interferon- γ assays that use similar select antigens (ESAT-6 and CFP-10) to differentiate *M. tuberculosis* infection from BCG vaccination in humans are in widespread clinical use (McNerney 2012). Although tests to detect and differentiate *M. bovis* infection from BCG vaccination would be important to facilitate the export of live cattle and cattle products, this is less relevant in settings where subsistence farming is the norm and where “test and cull” is not a control option.

A number of questions were raised at the meeting and priorities related to BCG vaccination research discussed. Additional field trials of BCG vaccine in cattle in different settings, such as exposure to different infection pressure or husbandry conditions with accompanying use of

DIVA tests are required to fully assess the efficacy and practicality of BCG vaccination against bTB in cattle. Key questions include whether maternal immunity interferes with effective vaccination of neonatal calves and if BCG vaccination will prevent onward transmission of bTB? Protection induced by BCG vaccination is not complete and vaccination should be integrated with other control measures that are feasible in the study setting, such as segregating bTB infected cows and feeding young calves born to infected mothers with pasteurised milk or with milk from non-infected cows. In some settings, it will also be important to minimize contact between cattle and infected wildlife reservoirs. A recent report from Great Britain recommended a thorough re-examination of different BCG vaccination models once DIVA tests have been refined and tested, with careful cost-benefit analysis that takes into account potential implications for International and United Kingdom trade (Godfray 2018).

BCG vaccination of other domesticated species

The dromedary camel (*Camelus dromedarius*) is extremely important for the livelihood of pastoral communities in arid areas of the world, including North Africa and the Middle East providing transportation, milk and meat. Camel milk and cheese are traditionally consumed raw, which combined with close physical human-animal contact, creates a public health concern for TB transmission. Camel TB has been reported in Egypt (Elmossalami 1971), the United Arab Emirates (Wernerly 2002, Kinne 2006), Pakistan (Zubair 2006), Nigeria (Ahmad 2018), and Australia (Manefield 1997), with both *M. tuberculosis* and *M. bovis* being isolated from diseased animals. Other camelid species, such as alpacas have also been noted to be susceptible (Godfray 2018).

For TB surveillance and diagnosis in camels there is a need to develop more sensitive and specific tests. At the workshop, studies were planned to assess the TB and brucellosis prevalence in camels from North Africa and the Middle East. BCG efficacy against TB in camels has not yet been evaluated. Collaborative TB prevalence and BCG vaccine efficacy studies could potentially be conducted in domestic camel herds in Israel, the Palestinian Authority, Saudi Arabia, Egypt and Morocco. Such an assessment could ascertain if BCG vaccination reduces TB disease rates and the presence of *M. bovis* in unpasteurized camel milk. Since camel brucellosis is a concomitant concern, *Brucella* prevalence and vaccination efficacy could be determined at the same time. These surveillance and intervention studies will also provide a platform to determine the burden of other diseases, such as parasitic infections, improving the health and well-being of highly prized and culturally significant camel herds.

BCG vaccination of wild animals

The requirements for the vaccination of wildlife *M. bovis* reservoirs differ from those of domestic animals in that it would be preferable if wildlife could receive a single vaccination dose and, for practical purposes, if the vaccine could be self-administered via an oral bait. Vaccination would mainly aim to decrease TB transmission among wildlife and spread to domestic animals. The use of bait BCG vaccines for possums in New Zealand and badgers in Ireland resulted in significant TB protection (Tompkins 2009, Gormley 2017). Oral BCG vaccination of wild boar and deer also induced protection against challenge with *M. bovis* (Gortazar 2014, Nol 2008). To date, BCG vaccination has been shown to be safe in all animal species tested (Perrett 2018, Murphy 2008). Oral vaccination of wildlife could be a useful tool for control of TB in wildlife and studies are required to optimize the dose and bait formulations, and to develop delivery systems that prevent uptake by non-target species such

as cattle where BCG bait consumption could result in the animal subsequently testing positive using a traditional TST.

Within South Africa, *M. bovis* infection has been reported in wildlife populations for nearly a century (Paine 1928, Jolles 2005). It is currently endemic in the Greater Kruger National Park Complex and the Hluhluwe-iMfolozi Park (Michel 2009, Hlokwe 2011), as well as several private farms and conservancies (Hlokwe 2016, South African Department of Agriculture, Forestry and Fisheries annual disease reports). African buffaloes are well known maintenance hosts of TB (Michel 2012) and play an important role in TB spillover to other wildlife species (Michel 2006), as well as “spill-back” to domestic cattle (Musoke 2015). Some rare and endangered species have been recently added to the list of TB susceptible species, including white rhinoceros (*Ceratotherium simum*), black rhinoceros (*Diceros bicornis*) Miller 2017, Miller 2015) and African wild dog (*Lycaon pictus*) (de Klerk-Lorist LM personal communication).

Since neither “test and cull” nor treatment are viable options in the majority of wildlife species, the only realistic alternative is vaccination. Studies in the African buffalo (*Syncerus caffer*) would be important to determine whether BCG vaccination offers herd-level protection against bTB in free ranging African buffalo populations. Preliminary studies in semi-free ranging buffalo have been disappointing, but various factors such as the age of the animals, the vaccination route, the challenge dose and grazing limitations could have contributed to the negative outcome (de Klerk 2010). More buffalo studies are important since they represent an important wildlife TB reservoir, with spill-over to other key species. Their limited dispersion should also facilitate vaccine delivery and monitoring of herd-level impacts.

Conclusion

Considering the human disease burden, the economic cost of livestock disease and the negative conservation impact resulting from bTB, judicious use of BCG vaccination in settings where “test and cull” is not an option requires urgent consideration. Collecting accurate surveillance data is important to help prepare field sites for future intervention trials.

Research on optimal BCG formulations and delivery methods should be advanced by addressing strain composition, dosage and administration method, as well as vaccine supply.

We are endeavoring to develop two animal BCG research programs; one focused on zoonotic disease risk in domestic camelids, and the second on the conservation of iconic African wildlife, focusing on the African buffalo and relevant “spill-over” species.

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Table 1. Bovine TB knowledge gaps and research needs identified at the Jerusalem workshop

Knowledge gap	Research need
<p>Poorly quantified prevalence of <i>M. bovis</i> infection and disease in humans, cattle, camelids, buffaloes and other relevant wildlife</p>	<ul style="list-style-type: none"> - Existing bTB surveillance data provide local snapshots, but fail to provide a global overview of the situation - bTB and <i>M. bovis</i> infection surveillance systems (animals and humans) in the less developed parts of the world are poorly developed or dysfunctional - Poor communication between human and animal health branches of government limit the exchange of relevant surveillance data - African buffaloes and American bison are important bTB reservoirs; the contribution of water buffaloes in Asian settings is poorly documented - <i>M. bovis</i> infection is not restricted to bovines. It may be a significant problem in domestic camelids, but the prevalence is unknown - Good surveillance data are essential to prioritize intervention sites, especially if current BCG trials in cattle demonstrate success
<p>What to do when “test and cull” is not an option?</p>	<ul style="list-style-type: none"> - “Test and cull” is unfeasible in settings where it is not economically viable, where cultural or religious objections exist, or where wild animal reservoirs exist in protected species - Settings where infected cows are long-lived pose the greatest risk, since they could spread infection for prolonged periods of time. More studies should track the natural history of disease and epidemic spread of bTB in settings where infected animals cannot be culled.
<p>Use of BCG to reduce bTB in domestic animals and wildlife</p>	<ul style="list-style-type: none"> - Proof of principle studies demonstrated significant bTB protection in cattle and wildlife, such as possums in New Zealand and badgers in Great Britain/Ireland - BCG has shown good protection against TB in humans and animals, but large scale studies in animals are lacking and few studies have investigated the use of oral BCG in problematic wildlife reservoirs - Novel BCG formulations and pragmatic delivery methods require careful consideration in relevant animal species - Limited research has investigated how <i>M. bovis</i> infection spreads within local ecosystems and how this can be contained. The conservation value of BCG vaccination in iconic wildlife species such as the African buffalo, and spill-over carnivore species such as lions and African wild dogs has not been considered.

bTB – bovine tuberculosis, mostly caused by *Mycobacterium bovis*; TB – tuberculosis, mostly caused by *M. tuberculosis*; BCG – *M. bovis* bacille Calmette-Guerin