BCG vaccination reduces bovine tuberculosis transmission, improving prospects for elimination

Science

Fromsa, Abebe; Willgert, Katriina; Srinivasan, Sreenidhi; Mekonnen, Getnet; Bedada, Wegene et al

https://doi.org/10.1126/science.adl3962

RESEARCH ARTICLE SUMMARY

VACCINATION

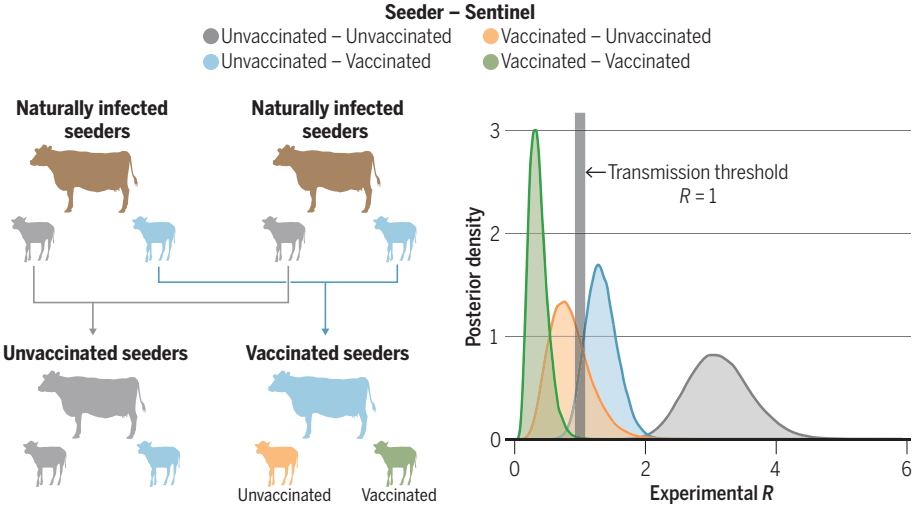
BCG vaccination reduces bovine tuberculosis transmission, improving prospects for elimination

Abebe Fromsa†, Katriina Willgert†, Sreenidhi Srinivasan†, Getnet Mekonnen, Wegene Bedada, Balako Gumi, Matios Lakew, Biniam Tadesse, Berecha Bayissa, Asegedech Sirak, Musse Girma Abdela, Solomon Gebre, Tesfaye Chibssa, Maroudam Veerasami, H. Martin Vordermeier, Douwe Bakker, Stefan Berg, Gobena Ameni, Nick Juleff, Mart C. M. de Jong, James Wood, Andrew Conlan*, Vivek Kapur*

INTRODUCTION: Bovine tuberculosis (bTB) poses a substantial global threat to animal health, food security, and human well-being. Although proven effective in many high-income countries, the traditional test-and-slaughter approach for bTB control is expensive and impractical for socioeconomic reasons in many regions where the disease remains endemic. This has necessitated a need for alternative bTB control strategies, with Bacille Calmette-Guérin (BCG) vaccination presenting a promising option. However, the effectiveness of BCG in controlling bTB by reducing onward transmission remains unclear. This study investigated both the direct and previously unexplored indirect effects of BCG vaccination on bTB transmission in cattle, thereby providing key missing insights for control.

RATIONALE: Traditional vaccine efficacy evaluations cannot measure the impact of vaccination on reducing onward transmission from infected individuals. This mode of action of vaccination is critical for the evaluation of BCG in cattle because the primary effect is to reduce the extent and rate of progression of lesions rather than to provide sterilizing protection. Our study addresses this gap by performing a natural transmission experiment with bTB in cattle, using a crossover design approach. This method enabled a more realistic and robust assessment of BCG's true impact on bTB transmission, quantifying both the direct efficacy of BCG as well as its effect on reducing transmission. We developed a mechanistic transmission model to explore the potential of using BCG vaccination in Ethiopia, where the transmission risk of bTB varies considerably between herds and the relatively infrequent trading of animals is projected to contribute to a gradual yet substantial increase in prevalence.

RESULTS: The natural transmission study showed a 74% reduction in bTB transmission [95% credible interval (CrI): 46 to 89%] in vaccinated as compared with unvaccinated animals.



Quantifying BCG vaccination's total efficacy against bovine tuberculosis. Sentinel calves, both BCG-vaccinated and unvaccinated, were exposed to seeder cattle to measure direct efficacy through IGRA-conversion times over 12 months. Subsequently, these sentinels were used to determine BCG's indirect transmission-reducing effects, and the results helped inform development of models for evaluating TB elimination strategies.

This substantial indirect effect of BCG vaccination exceeded the observed direct protection against infection (58%; 95% CrI: 34 to 73%), and the combined effects translated to a total vaccine efficacy of 89% (95% CrI: 74 to 96%).

Vaccinated animals exhibited substantially lower total visible lesion scores compared with unvaccinated controls, which is consistent with the notion that BCG vaccination reduces disease severity and potentially infectiousness.

A stochastic metapopulation transmission model, calibrated with data from Ethiopia, suggests that routine calfhood BCG vaccination has the potential to prevent the predicted expansion of bTB in dairy herds and bring the population average reproduction ratio below 1 within as few as 10 years, resulting in a substantial decrease in predicted bTB prevalence as compared with baseline scenarios without vaccination. The results highlight the critical importance of the combined direct and indirect effects of BCG vaccination in enabling bTB elimination.

The findings suggest that BCG vaccination represents an important tool for bTB control, particularly in resource-limited settings where traditional methods are impractical. The results also suggest that achieving elimination through vaccination alone would require a long-term commitment, as the full benefits may take decades to be realized. Our studies highlight a need for further research on the duration of efficacy, including the potential for extending protection through revaccination, as well as the impact on cross-species transmission.

CONCLUSION: Our study demonstrates remarkable and previously unrecognized indirect effects of BCG vaccination on bTB transmission, extending beyond its direct protective effect. Scenario analyses with mechanistic models for transmission in Ethiopia suggest that implementation of BCG vaccination may enable effective bTB control and progress toward elimination. Moreover, these findings suggest that BCG may provide an effective method of control in resource-limited settings where current test-and-slaughter approaches are unfeasible. Lastly, the crossover trial design incorporating natural transmission provides a general framework for studying other vaccines and interventions aimed at reducing onward transmission of TB, with broad applicability to other infectious diseases of animals, including humans. ■

The list of author affiliations is available in the full article online.
***Corresponding author. Email: vxk1@psu.edu (V.K.); ajkc2@cam.ac.uk (A.J.K.C.)**
†These authors contributed equally to this work.
Cite this article as: A. Fromsa *et al.*, *Science* 383, eadl3962 (2024). DOI: 10.1126/science.adl3962

READ THE FULL ARTICLE AT
https://doi.org/10.1126/science.adl3962

RESEARCH ARTICLE

VACCINATION

BCG vaccination reduces bovine tuberculosis transmission, improving prospects for elimination

Abebe Fromsa[1,2]†, Katriina Willgert[3]†, Sreenidhi Srinivasan[4,5,6]†, Getnet Mekonnen[7], Wegene Bedada[7], Balako Gumi[1], Matios Lakew[7], Biniam Tadesse[7], Berecha Bayissa[1]‡, Asegedech Sirak[7], Musse Girma Abdela[1], Solomon Gebre[7], Tesfaye Chibssa[7], Maroudam Veerasami[8], H. Martin Vordermeier[9], Douwe Bakker[4,10,11], Stefan Berg[9]§, Gobena Ameni[1,12], Nick Juleff[13], Mart C. M. de Jong[14], James Wood[3], Andrew Conlan[3]*, Vivek Kapur[4,5]*

Bacillus Calmette-Guérin (BCG) is a routinely used vaccine for protecting children against *Mycobacterium tuberculosis* that comprises attenuated *Mycobacterium bovis*. BCG can also be used to protect livestock against *M. bovis*; however, its effectiveness has not been quantified for this use. We performed a natural transmission experiment to directly estimate the rate of transmission to and from vaccinated and unvaccinated calves over a 1-year exposure period. The results show a higher indirect efficacy of BCG to reduce transmission from vaccinated animals that subsequently become infected [74%; 95% credible interval (CrI): 46 to 98%] compared with direct protection against infection (58%; 95% CrI: 34 to 73%) and an estimated total efficacy of 89% (95% CrI: 74 to 96%). A mechanistic transmission model of bovine tuberculosis (bTB) spread within the Ethiopian dairy sector was developed and showed how the prospects for elimination may be enabled by routine BCG vaccination of cattle.

Bovine tuberculosis (bTB) is an economically important disease of livestock with the potential for zoonotic transmission (*1*). Statutory programs for elimination currently rely on intensive test-and-slaughter of animals that react to tuberculin, which provides a measure of exposure but not necessarily of infection. Test-and-slaughter based elimination programs have been successful in many countries including Australia (*2*) and the United States (*3*) but are unfeasible socioeconomically in most low-and-middle income countries (LMICS), where the disease is uncontrolled. Test-and-slaughter also continues to place considerable economic pressures in countries such as the United Kingdom (*4*), Ireland (*5*), and New Zealand (*6*), where the disease persists in cattle and wildlife despite intensive efforts. The development and deployment of vaccines such as Bacillus Calmette-Guérin (BCG) has thus long been seen as a solution for accelerating control and the ultimate elimination of bTB across the globe (*4*). However, the use of BCG vaccination as a supplement, rather than an alternative, poses economic challenges because of the ongoing costs associated with test-and-slaughter and the potential for BCG to undermine the effectiveness of existing bTB control programs (*7*, *8*).

Surveillance for bTB relies predominantly on tuberculin testing, a method that detects delayed hypersensitivity reactions to purified protein derivatives (PPDs, also known as tuberculins) from specific *Mycobacterium* species. The standard approach internationally is the skin test, in which the response is assessed by the size of the swelling at the injection site. Complementing this, interferon-gamma release assays (IGRAs) offer a blood test alternative that use the same tuberculins but avoid the issue of desensitization that may be observed in animals repeatedly exposed to tuberculins. Animals vaccinated with BCG yield higher rates of false positive reactions to tuberculin in both formats of the test (*9*), which is the primary reason it has not yet been licensed for use in domestic livestock. Recent developments in diagnostic tests with defined antigens (ESAT-6, CFP10, Rv3615c, and others) provide a promising avenue for the detection of infection in vaccinated animals (DIVA) testing (*10*–*13*) and potentially enable the use of BCG vaccination without compromising the ability to assess freedom from infection. These considerations are perhaps less relevant for endemically infected LMICs with major cattle populations throughout the world, such as Ethiopia or India, where the high but variable prevalence of infection within herds (*14*–*17*) makes the introduction of test-and-slaughter economically prohibitive and the use of vaccines more attractive. Intensification of farming in these emerging dairy markets poses the risk that cattle movements will fuel a parallel increase in the prevalence of bTB. For instance, the animal-level prevalence in Ethiopia has been recently estimated at 5.8% [95% confidence interval (CI): 4.5 to 7.5%] (*18*), but with the prevalence within some heavily infected herds approaching 100% test positivity, this burden could dramatically increase.

Although DIVA tests are being validated to pave the way for vaccination programs, the efficacy of BCG remains a subject of debate. Recent meta-analyses estimate its direct protection against infection to be as low as 18% [95% credible interval (CrI): 11 to 24%] in experimental studies and 61% (95% CrI: 40 to 74%) in natural transmission models (*19*). This low and variable primary direct efficacy (VE_D) is consistent with BCG's performance in human populations (*20*). However, in the context of bTB control, these direct effects only partially capture the vaccine's impact on transmission dynamics. The primary claim for BCG is its ability to decelerate disease progression and minimize lesions. In closed herds with high vaccination rates, the total vaccine efficacy (VE_T) also encompasses an indirect effect (VE_I), which represents the reduced transmission rate from vaccinated, infected animals to others (*21*). This indirect effect is increasingly recognized as a critical factor for assessing BCG's overall effectiveness in field conditions (*19*).

Existing literature primarily calculates BCG's efficacy on the basis of the number of positive animals in both vaccinated and control groups at a fixed end point, often using the postmortem presence of visible lesions or culture confirmation as this end point (*19*), and in one instance, by using multiple antemortem diagnostic tests (*22*). Such end point–based estimates are problematic for chronic diseases such as bTB in that they are highly sensitive to the force of infection within the study population and the duration of exposure (fig. S1), hence making them unsuitable for predicting the effectiveness of vaccines or other interventions under field conditions. Hence, to accurately gauge BCG's utility in controlling bTB, there is a critical need to focus on its efficacy in reducing transmission rates. This is particularly crucial given the consistent evidence that BCG vaccination slows down disease progression and reduces lesion extent (*19*), implying that vaccinated animals, if infected, are less infectious. Therefore, the relevant metric for control is not the traditionally reported

[1]Aklilu Lemma Institutes of Pathobiology, Addis Ababa University, Addis Ababa, Ethiopia. [2]College of Veterinary Medicine and Agriculture, Addis Ababa University, Bishoftu, Ethiopia. [3]Disease Dynamics Unit, Department of Veterinary Medicine, University of Cambridge, UK. [4]Huck Institutes of Life Sciences, The Pennsylvania State University, University Park, PA, USA. [5]Department of Animal Science, The Pennsylvania State University, University Park, PA, USA. [6]The Global Health Initiative, Henry Ford Health, Detroit, MI, USA. [7]Animal Health Institute, Sebeta, Ethiopia. [8]CisGen Biotech Discoveries, Chennai, India. [9]Animal and Plant Health Agency, Weybridge, UK. [10]Technical Consultant and Independent Researcher, Lelystad, Netherlands. [11]Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad Complutense, Madrid, Spain. [12]Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, United Arab Emirates University, United Arab Emirates. [13]The Bill & Melinda Gates Foundation Seattle, WA, USA. [14]Quantitative Veterinary Epidemiology Group, Wageningen UR, The Netherlands.
***Corresponding author. Email: vxk1@psu.edu (V.K.); ajkc2@cam.ac.uk (A.J.K.C.)**
†These authors contributed equally to this work
‡Present address: National Veterinary Institute, Bishoftu, Ethiopia
§Present address: Bernhard Nocht Institute for Tropical Medicine, 20359 Hamburg, Germany

end-point estimate but rather the vaccine's efficacy in reducing transmission rates, especially so in closed herds with potential for high vaccination coverage (*23*). The comprehensive effects of vaccines, extending beyond mere reduction in susceptibility, have been previously explored for acute viral infections (*23*, *24*). However, their application to chronic bacterial infections, including bTB, are notably lacking. This knowledge gap is particularly relevant given the complex nature and extended course of chronic infections, for which an understanding of the full effect of vaccines, including impact on onward transmission, is key.

To quantify the efficacy of both the direct and indirect modes of action of BCG to reduce transmission in cattle, we carried out a natural transmission study under controlled conditions (Fig. 1). In brief, four experimental groups of sentinel animals were included in the study, each exposed to different types of seeder animals across two distinct phases, with each phase lasting for 12 months of contact. In phase I, two groups (groups 1 and 2) functioned as biological replicates. Each group included approximately equal numbers of seeder (~34) and sentinel animals, divided equally between vaccinated (~17 animals) and unvaccinated controls (~17 animals). Following an initial 2 months after vaccination, these sentinels were introduced to a separate barn, joining older test-positive seeder animals sourced from local Ethiopian dairy herds. In phase II, sentinel animals from phase I were reassigned into two groups on the basis of their vaccination status: vaccinated $n = 32$ and unvaccinated $n = 32$. These animals then served as seeder animals for a new set of approximately 34 sentinel calves (similar to the arrangement during phase I). We next developed a mechanistic transmission model calibrated using estimates of the within-herd reproduction ratio, R_0, and empirical cattle movement data from four regions of Ethiopia, to explore the potential for control of bTB in LMIC settings through routine BCG vaccination.

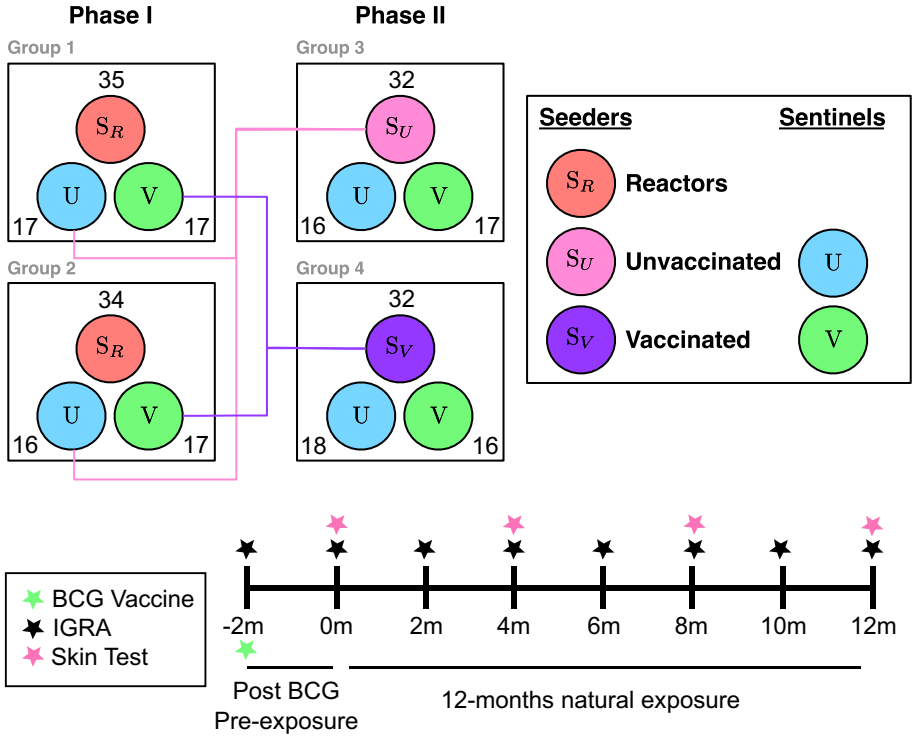


Fig. 1. Schematic representation of study design. For phase I, sentinel animals (calves) were acclimatized for about 2 weeks after recruitment, and then they were randomized (double-blind lottery system) into control (U) and vaccinate (V) experimental groups. At 2 months after vaccination, blood was drawn for IGRA, and a skin test was conducted to confirm vaccine response. After confirmation of vaccine response, two sets of ~34 adult infected (CCT and IGRA+) seeders were housed with a target of 17 unvaccinated and 17 BCG-vaccinated sentinel calves (2 to 6 months of age) in each group (group 1 and 2) with a range of between 16 to 18 animals in practice after the loss of animals after vaccination but before exposure. A total of 5, 4, 4, and 3 animals in total were removed early from groups 1, 2, 3, and 4, respectively. The majority of removals in each group (except for group 3) were seeders consisting of 3/5, 3/4, 1/4, and 2/3, respectively. During the exposure period of 1 year, blood was drawn for IGRA every 2 months, and the skin test was conducted every 4 months. The surviving sentinel animals from phase I were then used as seeders for two new experimental groups (groups 3 and 4) with a fresh set of sentinel calves, which were then followed with the same schedule of tests for a second 12-month period of natural exposure.

Trajectories of cellular immune responses in BCG-vaccinated cattle

After acclimatization and before vaccination, sentinel animals' negative bTB status was established with IGRA using PPDs from avian (A) and bovine (B) mycobacteria as the stimulating antigens. All sentinel animals entering the experiment showed no signs of infection and tested negative with no significant difference observed between responses elicited by controls and vaccinates to PPD (B – A) in IGRA before BCG vaccination (fig. S2A). At the eighth week after BCG vaccination, and before exposure to seeder herds, both vaccinated and control calves were subjected to skin and IGRA blood tests with bovine and avian PPDs to confirm vaccine response.

The infection status of sentinel animals within each experimental group was then monitored using the same sequence of skin tests (bovine, avian, and DIVA antigens) at four-month intervals, with whole-blood samples taken every 2 months for IGRA assays using the same set of antigens (Fig. 1). In phase II, the sentinel animals exposed to naturally infected comparative cervical tuberculin (CCT) test–positive animals during phase I were used as seeder animals in phase II, split according to vaccination status (*25*). Thus, by comparing the rates of transmission experienced within the four experimental groups, we were able to directly compare the relative infectiousness of vaccinated and unvaccinated animals. Transmission rates were inferred by estimating a discrete-time stochastic chain-binomial model (*26*, *27*) according to logical infection histories imputed from the diagnostic test results (Fig. 1).

Given the chronic nature of bTB and lack of reliable antemortem correlates of infectiousness, animals are assumed to be infected, and potentially infectious, from their first positive test through the duration of observation. Although the inferred transmission rates based on test positivity are influenced by the sensitivity of the diagnostic assay, for the purposes of estimating vaccine efficacy, the baseline transmission rate may be treated as a nuisance parameter because the efficacy depends only on the relative magnitude of transmission rates between different treatment groups and not on the absolute values. Traditional tuberculin-based tests are ill-suited for this purpose because they reduce specificity for BCG-vaccinated animals, thereby inflating apparent transmission rates. By contrast, DIVA tests with peptide-based defined antigens offer more precise estimates with the key assumption that they perform equivalently in both vaccinated and control animals. Consistent with this assumption, even though the response magnitude for DIVA tests is generally lower than for tuberculin tests, and though DIVA tests have higher specificity for BCG-vaccinated animals, prior evidence from multiple experimental trials

suggests that the commonly used categorical cutoffs do not result in performance differences or misclassification of infected animals (*28*). For this study, we used antigens developed for the defined skin test (DST) in both a skin and IGRA format. Owing to the risk of desensitization of animals from repeated exposure to antigens, skin testing was carried out at a lower frequency (four-month intervals) compared with that for IGRA. Given the higher temporal resolution, we focus on the DST1 (IGRA) results and compare estimates of vaccine efficacy with the more uncertain DST 10 (skin test) results.

Estimates of direct and indirect efficacy of BCG

DST-based IGRA test history was used to calculate the baseline experimental reproduction ratio (defined as the number of new infections expected in which a single infectious individual is held in contact with a fully susceptible population for a 12-month period), which was estimated to be 3 (95% CrI: 2 to 4%). The attack rate for both vaccinates and controls was consistent across all the experimental groups except for the group with vaccinated seeders (Group 4), indicating that high indirect protection of BCG reduced the infectiousness of vaccinates (fig S1). This effect is reflected in an estimated indirect vaccine efficacy for reduction in infectiousness (Fig. 2C) of VE_I = 74% (95% CrI: 46 to 89%); this is higher than the estimated direct protection of VE_D = 58% (95% CrI: 34 to 73%), with a 86% posterior probability that VE_I is greater than VE_D. Taken together, this corresponds to a total vaccine efficacy of BCG to reduce transmission of bTB of 89% (95% CrI: 74 to 96%). Estimates from the DST 10 (skin test) are consistent but more uncertain, which is in line with the lower temporal resolution of this data (Table 1).

The practical importance of the indirect vaccine effect is reflected in the pairwise experimental reproduction ratios (Fig. 2B and Table 2), which show that the direct and indirect effects of BCG, when considered alone, are not sufficient to prevent transmission within the experimental system, with posterior probability that R is less than the critical threshold value of 1 of only 0.075 for vaccinated sentinels placed in contact with unvaccinated seeders, rising to 0.72 for the combination of vaccinated seeder animals and unvaccinated sentinels (Table 2). By contrast, the posterior probability that $R < 1$ is 1.0 for the combination of vaccinated sentinel animals and seeders, implying that both direct and indirect modes of action of BCG could achieve elimination in our experimental system. This has important consequences for the utility and monitoring of BCG vaccination policies in endemic settings because the full protective benefits may take multiple generations to accrue.

The results of our investigations also corroborate the findings from previous studies on BCG vaccination's partial protective effects and lesion reduction in cattle (*19*). Although lesions were still observed in vaccinated animals, the total visible lesion scores were notably

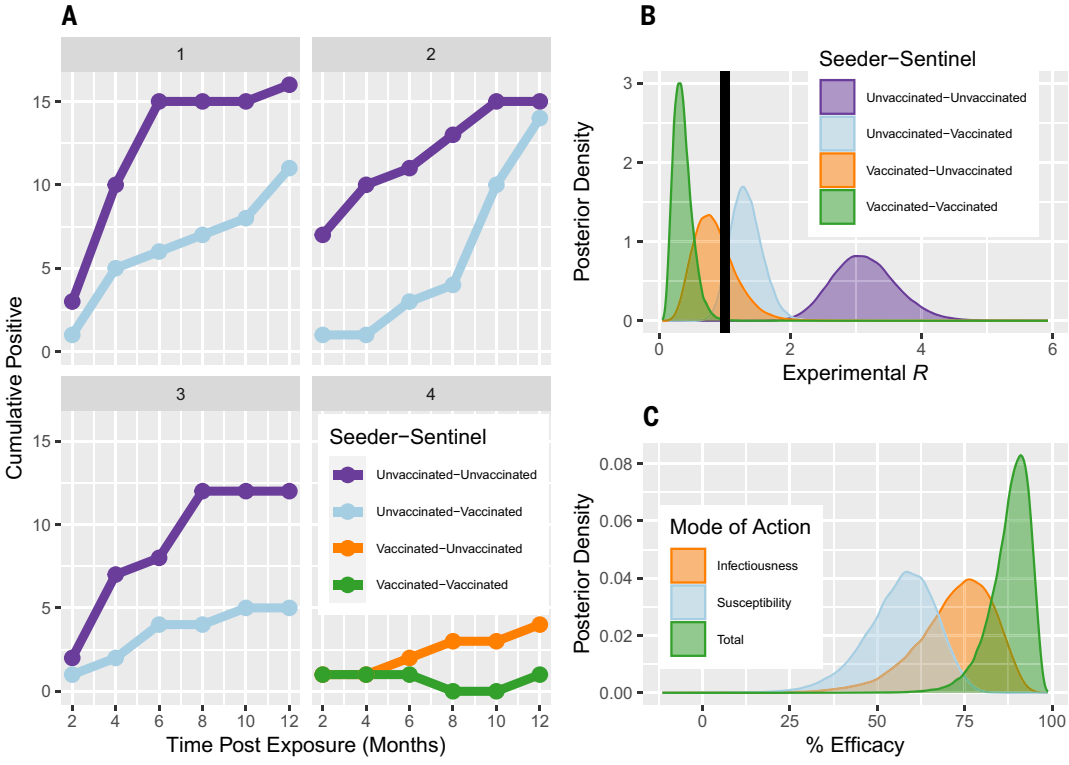


Fig. 2. The two modes of action of BCG vaccination in cattle. (**A**) The infection history for unvaccinated (purple, orange) and vaccinated (light blue, green) animals within each experimental group (1 to 4) as represented by the cumulative number of animals that have ever tested positive by IGRA (DST1) and have survived to that time point. These curves represent the number of sentinel animals assumed infectious within each group at the test date, reflecting the animals removed on welfare grounds, as well as those that have tested positive. The lower attack rate in group 4, in which all seeder animals were vaccinated, demonstrated the reduced infectiousness of these animals compared to that of the other groups with unvaccinated seeders. (**B**) The estimated transmission rate between the different combinations of vaccinated and unvaccinated sentinel and seeder animals can be expressed as an experimental reproduction ratio R, defined as the expected number of new infections in a fully susceptible population of the given sentinel type when exposed for a duration of 1 year to the respective seeder type. Posterior distributions for the four combinations are presented with the threshold value of R = 1 indicated by the vertical line. Only the combination of vaccinated sentinels and seeders within our experimental unit shows a high posterior probability that $R < 1$, which increases from 0.72 to 1.0 with vaccination of both seeder and sentinel groups (**C**). Posterior estimates for the vaccine efficacy of BCG with respect to reducing infectiousness (VE_I, orange), reducing susceptibility (VE_D, light blue), and the total efficacy (VE_T, green). This total efficacy represents a combination of both direct (reducing susceptibility) and indirect (lowering infectiousness) effects of the vaccine.

Table 1. Estimated vaccine efficacy for BCG in cattle by mode of action.

Diagnostic test	End-point efficacy	Transmission rate efficacy		
	Total efficacy (95% CI)	Direct efficacy (VE_D) (95% CrI)	Indirect efficacy (VE_I) (95% CrI)	Total efficacy (VE_T) (95% CrI)
DST 1 (IGRA)	39 (22 to 54)	58 (34 to 73)	74 (46 to 89)	89 (74 to 96)
DST 10 (skin)	40 (19 to 57)	46 (14 to 66)	67 (39 to 87)	82 (55 to 94)
Visible lesions	25 (1 to 47)			

Table 2. Estimated experimental reproduction ratios by vaccination type of sentinel and seeder animals.

Seeder Sentinel pair	*R* (1-year contact) (95% CrI)	Posterior probability that $R < 1$
Unvaccinated-Unvaccinated	3.1 (2.3 to 4.1)	0.0
Unvaccinated-Vaccinated	1.3 (0.9 to 1.8)	0.075
Vaccinated-Unvaccinated	0.8 (0.4 to 1.5)	0.72
Vaccinated-Vaccinated	0.3 (0.1 to 0.7)	1.0

lower compared with those of the control group across both seeder (phase 1 sentinels) and sentinel animals (fig. S2). Given the study's design constraints, which necessitated retaining phase I sentinel animals as seeders for phase II, it was not possible to obtain end-point efficacy estimates based solely on visible lesion scores. However, when pooling all sentinel animals from phase II groups 3 and 4, we observed a 1-year end-point efficacy of 25% (95% CI: 1 to 47%) (Table 1), which is consistent with earlier meta-analysis estimates (*19*). Although BCG's inability to fully prevent infection could raise questions about the efficacy estimates being influenced by reduced DIVA antigen detection sensitivity in vaccinated animals, the study design and results mitigate this concern. Specifically, if reduced test sensitivity among vaccinated animals were influencing the results, we would not expect to see the marked reduction in the attack rate among unvaccinated controls that was also seen in group 4 (Fig. 2A).

Prospects for elimination of bTB by BCG vaccination

To explore the consequences of implementing BCG vaccination in a relevant context, we assessed the feasibility of eliminating bTB from the Ethiopian dairy sector through routine vaccination alone. Using tuberculin skin-testing data from the Ethiopia Control of Bovine Tuberculosis (ETHICOBOTS) project (*15*, *16*) and historical data from the Ethiopia's Animal Health Institute (*14*), we estimated within herd reproduction ratios (R_0)—defined as the expected number of new infections when a single infectious individual is introduced into a fully susceptible population—ranging from 1.3 to 17.3 (Fig. 3B), with a population average of 3.26 (95% CrI: 2.7 to 4.0%). Given that cattle rarely show clinical signs of bTB during the early stages of disease, cattle trade can facilitate long-distance transmission in the absence of systematic herd testing. Consequently, we considered cattle movements as the primary mode of transmission between herds. Directed exponential random graph models (ERGMs) were used to estimate new generative models for the frequency and pattern of cattle movements between herds in Ethiopia previously collected from three emerging dairy markets (*29*).

To predict the likely spread of bTB on the basis of estimated networks of movements and to assess the potential benefits of vaccination, a stochastic metapopulation transmission model was developed with four possible states: susceptible (S), infected (I), vaccinated (V), and infected vaccinated (I_V). Three transmission scenarios were simulated and were motivated by the three regions around Gondar, Mekelle, and Hawassa for which movement data were available. For each scenario, a synthetic population was simulated with initial herd sizes sampled from the recorded herd sizes in Ethiopia and initial herd-level prevalence sampled with point estimates from (*15*) (18.0% in Gondar, 38.9% in Mekelle, and 10.5% in Hawassa). The intrinsic within-herd R_0 was assumed to vary between herds and was sampled from our estimates. Affected herds were initialized at the endemic equilibrium and simulated forward for 50 years.

The baseline scenarios without vaccination (Fig. 3C) demonstrate the scale of the potential risk posed by cattle trading, with a predicted increase in the animal-level prevalence across all scenarios to an endemic level of ~50%. However, this emergence is predicted to be slow—mediated by the relatively infrequent trades of animals—and to require the full 50-year span of our simulated scenario. This relatively slow progression, in contrast to the rapid potential for cattle-to-cattle transmission within herds as shown in our natural challenge system, offers a window of opportunity for vaccination or indeed for interventions targeted on the cattle-movement networks (*29*).

The effectiveness of vaccination as a control measure for bTB was then evaluated by comparing the current baseline scenario with scenarios in which all newborn calves are vaccinated (Fig. 3C). The potential waning of vaccine protection over time was not considered, and it was assumed that animals would be revaccinated as needed to maintain levels of protection. To illustrate the importance of the additional indirect protection afforded by BCG, we compared two vaccine scenarios in which animals are protected only by a direct effect or with the combined direct and indirect effects (sampled from our posterior estimates). Our model suggests that with the estimated total vaccine efficacy of 89% (95% CrI: 74 to 96%), calfhood BCG vaccination can prevent the predicted expansion of bTB through the dairy sector in Ethiopia, bringing the population average *R* below 1 within 10 years of deployment. However, the path to elimination is slow, with herd prevalence still above the international officially TB-free level of <0.1% annually through the 50-year program simulation. In common with our experimental system, this impact can only be achieved as a result of both the direct and indirect effects of BCG (Fig. 3C).

Implications for the deployment of BCG in the field

Our scenario analysis demonstrates the challenges of controlling a chronic infection such as bTB, in which infected animals potentially remain infectious for life. Without the chance of recovery, the scale by which transmission is measured and control is manifested is determined by the life expectancy of the host. Through this lens, even under the most favorable circumstances, routine BCG vaccination as the only control measure is expected to take decades to eliminate bTB infection. Even so, in endemic

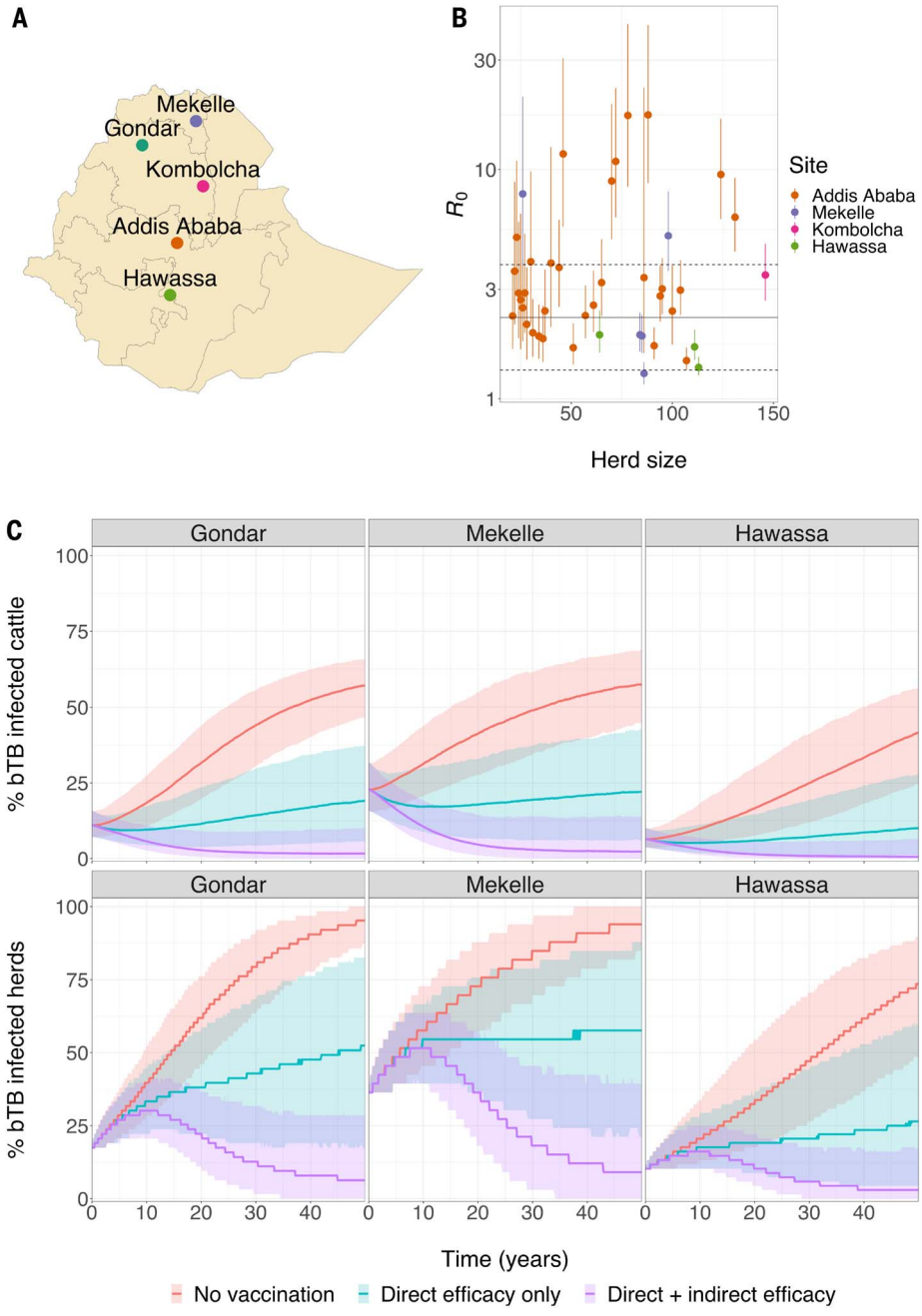


Fig. 3. Scenario analyses for the prospective use of cattle vaccination within the Ethiopian dairy sector. (**A**) Within-herd rates of transmission of bTB were calibrated with herd level prevalence data collected from herds in the dairy belt surrounding Addis Ababa and emerging markets in Hawassa, Mekelle, and Kombolcha. Gondar, Hawassa, and Mekelle are the only regions for which we also have between-herd cattle movement data. (**B**) The wide range of estimated animal-level prevalence within herds (5.8 to 78%) corresponds to a similarly large heterogeneity in estimates of the basic reproduction ratio, R_0, with no clear associations with either geography or herd size. (**C**) Cattle movement models estimated data from Gondar, Mekelle, and Hawassa were used to project how the prevalence of bTB might be expected to evolve over the next 50 years. The projected median (solid line) and 95% prediction intervals (shaded area) for the percentage of herds infected with bTB for this baseline scenario (no vaccination, pink) are compared with scenarios with routine vaccination coverage of all herds (with no assumed loss of immunity) with the estimated direct vaccine efficacy of 58% (95% CrI: 34 to 73% only) (light blue) and with the additional indirect efficacy of 74% (95% CrI: 46 to 89%) (purple).

settings, vaccination could play an important role in limiting spread and reducing prevalence such that more rapid measures such as test-and-slaughter or test-and-segregate can become economically tractable. The long-term commitment to vaccination necessary to see epidemiological impacts must be factored into economic cost-benefit analyses to support the business case for specific regions. These benefits are likely to vary considerably between settings depending on the impact of bTB on animal production and productivity, both of which are remarkably poorly characterized outside of a single study from Germany in 1970 (*30*).

The evidence for substantial indirect protection provided by BCG in calves contrasts with recent findings from field trials with badgers in Ireland (*31*), but it is consistent with a

smaller-scale trial within badger social groups in England (*32*). Because the evaluation of indirect vaccine effects in free-living animals—which depends on comparing the attack rates in subpopulations with varying levels of vaccine coverage (*21*)—is exceptionally challenging and typically only feasible once vaccines have been deployed at scale, the contradictory findings from the badger trials in England or Ireland are difficult to interpret in context. It is noteworthy that despite the widespread use of BCG vaccine globally, no similar indirect effects have been reported with respect to the transmission of human tuberculosis. The well-known geographic variation in BCG efficacy in human populations (*20*), more complex life history of infection, and the regular removal of infectious individuals through treatment (and mortality) would make such an effect more difficult to measure than in the managed settings of cattle production systems.

Despite the evidence for indirect effects of BCG in cattle and the promising implications of its use in accelerating bTB control programs, there are several limitations to our study. A key limitation is the relatively short duration of 1-year contact with infected animals as part of these controlled trials. This is relevant because experimental studies with a revaccination approach indicate that the duration of direct protection of BCG in cattle is limited to between 1 and 2 years (*33*). Hence, the high efficacy of BCG to reduced infectiousness that was seen in our trial may not be maintained for the average lifetime of a dairy cow in the herd, which is ~7 years in Ethiopia (*34*). Future investigations to assess the duration of indirect effects on infectiousness, as well as the potential to maintain or extend these effects through revaccination, remain key knowledge gaps, both of which will likely only be practical to measure once the vaccine is widely deployed in the field. Another limitation is that our current models do not account for cross-species transmission from other livestock held on the same premises as cattle, such as pigs, goats, and sheep; sympatric free-living wildlife species; or even humans—all of which may act as potential reservoirs of spillover infection to cattle. Future studies are needed to assess the potential benefits of cattle vaccination in the face of risk of cross-species transmission from other sympatric hosts (*35*). Lastly, our model only projects the expected increase in prevalence of bTB according to current patterns of movements and rates of transmission within dairy herds; intensification and changes in husbandry are likely to change both of these factors over such a long period that our scenarios should be considered only as projections to illustrate the potential benefits of vaccination rather than as forecasts.

The implications of our studies for populations with established surveillance and control programs, such as the United Kingdom, are more nuanced because the costs associated with bTB are dominated by the impacts on the test-and-slaughter program itself (*7*) rather than the prevalence of disease. Concern over the potential for perverse consequences of vaccination, given the evidence for limited direct protection and the compromised specificity of tuberculin diagnostics in vaccinated cattle, has been a major barrier for the willingness of statutory and governmental agencies to license BCG for use in cattle (*8*). These concerns should be somewhat alleviated by the results of our investigations, which show that the indirect protection afforded by BCG vaccination is at least equal to if not greater than the direct protection typically measured by efficacy studies.

Our studies suggest that the effectiveness of BCG in the field is likely to be greater and the risks lower than previously understood, and we provide key missing evidence of the potential benefits of a BCG vaccination–based approach to accelerate control of bTB in regions where the disease remains endemic.

Methods summary

Experimental study location and subjects

The study took place at the Animal Health Institute, located in Sebeta, Ethiopia, about 20 km southwest of Addis Ababa. The premises had three separate barns, each capable of accommodating 80 adult cattle, with approximately 2000 to 2500 m^2 of fenced outdoor space. In phase I, 68 naturally infected adult cattle were recruited as seeders animals for groups 1 and 2 of the transmission experiment. Sixty-eight Holstein-Friesian × Zebu crossbred male calves, age <3 months and tested negative for bTB, were recruited as naïve sentinels from low-prevalence herds split between the two experimental phases, with sentinel animals from phase I (groups 1 and 2) acting as seeder animals in the second phase (groups 3 and 4).

Testing procedures, antigens, and vaccination

Eight weeks after BCG vaccination, all sentinel animals were introduced to infected adult seeder herds. The seeders and sentinels were housed together in two separate barns during both phase I and phase II. Every four months, skin tests were conducted on sentinel animals using both PPDs and the recently developed DST antigens. Blood was drawn for IGRA every two months. Bovine (PPD-B) and avian (PPD-A) tuberculins were used as stimulating antigens in both skin and interferon-gamma (IFN-γ) release assays. A novel peptide cocktail representing the antigens ESAT-6, CFP-10, and Rv3615c was also utilized. We refer to the skin test format of the DST as DST10 and the IGRA as DST1 (corresponding to the units of antigen used respectively for each test). Full details of the IGRA and skin test protocols are provided in the supplementary materials. The calves of the vaccinate group were subcutaneously injected with 0.5 ml (1-4 × 106 CFU) of live BCG Danish Strain SSI 1331, while the controls received 0.5 ml of 0.9% normal saline (fig. S4).

Definition of logical infection histories and chain binomial model to estimate vaccine efficacy

For the purpose of analysis, the infectious status for each animal is imputed through the response to each of the candidate DIVA tests. For each variant of the DIVA test (DST1, DST10), we constructed a logical infection history where animals are considered to be infected—and potentially infectious—from the first positive test. The number of new infections per observation period is therefore calculated as the number of animals that switch diagnostic status within that interval.

From the imputed time series of infection states, we can estimate the rate of transmission to control and vaccinate animals, respectively, using a chain-binomial model (*27*, *36*) in which we assume that the rate of susceptible animals acquiring infection between two tests is constant and given by the number of infected (unvaccinated) animals I and infected vaccinates I_V at the earlier test, as follows

$$R(S \rightarrow I) = \beta \frac{(I + \varepsilon_I I_V)}{N}$$

where N is the total number of animals within the experimental group, β can be interpreted as the per capita transmission rate per infectious animal, and ε_I measures the reduction in infectiousness of vaccinated animals that become infected (relative to unvaccinated controls). Under these assumptions, the probability of transmission within a discrete time interval can be calculated and the number of new cases within that interval will be binomially distributed. As the probability of infection takes the same function functional form as the complementary log-log transformation, this chain-binomial model can be estimated using a generalized linear model (GLM) with binomial response (and complementary log-log link function). We used the rstanarm package (*37*) in R (*38*) to estimate the model within a Bayesian framework and obtain posterior estimates for the direct vaccine efficacy to reduce susceptibility ($1 - \varepsilon_S$), efficacy to reduce infectiousness ($1 - \varepsilon_I$), and average base-transmission rate (β) within the two experimental barns. Convergence was assessed using standard diagnostics (Rhat and effective sample size), and model fit was assessed using posterior predictive checks (specifically by forming Bayesian predictive P values for the proportion of zeros, maximum, and mean values).

Mechanistic transmission model for scenario analysis

A stochastic meta-population model with herds as the basic epidemiological unit was developed

to carry out scenario analyses for the potential impacts of BCG vaccination in Ethiopia and implemented using the R package SimInf (*39*). This compartmental model tracks four possible epidemiological states—susceptible (S), infected (I), vaccinated (V), and infected vaccinated (I_V)—and eight events corresponding to birth, transmission, and mortality (fig. S9). Full details of the model structure and calibration are presented in the supplementary materials along with raw data, R scripts, and model code (*40*).

Transmission rates within herds were calibrated from estimates of the basic reproduction ratio R_0 derived from tuberculin testing data from herds in five study sites across Ethiopia, including the dairy belt surrounding Addis Ababa and emerging markets in Gondar, Hawassa, Mekelle, and Kombolcha (*14*–*16*). Herds were assumed to be endemically infected with the value of R_0 inferred from the quasi-stationary distribution of the susceptible-infected-susceptible (SIS) model (*41*). To adjust for the imperfect sensitivity and specificity of tuberculin testing, the true prevalence of infection within herds was inferred with a latent class model (*42*, *43*). The model was implemented in Stan and analyzed in R with the RStan package (*37*). Transmission between herds was assumed to be mediated by cattle movements. Using samples of movement records collected alongside tuberculin testing data for a subset of herds in Ethiopia, we used ERGMs to construct generative models for synthetic movement networks with the same statistical properties as the measured movement networks from Gondar, Hawassa, and Mekelle (*44*, *45*).

The initial number of animals in a herd was randomly sampled from the recorded herd sizes taken from this subset of our study populations of herds with movement data. The population size of a herd was assumed to be constant, and the birth rate in a herd was balanced against the mortality rate and the average movement rate of cattle in the herd. The effectiveness of vaccination as a control measure for bTB was evaluated by comparing scenarios with no vaccination with those in which all newborn calves were vaccinated. For all scenarios, we sampled vaccine efficacy from the posterior estimates derived from our natural transmission study and carried out a further sensitivity analysis to the reduction in infectiousness (ε_I) for values of 0, 25, and 50%. The range of vaccination scenarios explored is summarized in Fig. 3, and outputs were simulated from 1500 replicates of the stochastic model.

REFERENCES AND NOTES

1. F. Olea-Popelka *et al*., Zoonotic tuberculosis in human beings caused by Mycobacterium bovis-a call for action. *Lancet Infect. Dis.* **17**, e21–e25 (2017). doi: 10.1016/S1473-3099(16)30139-6; pmid: 27697390
2. S. J. More, B. Radunz, R. J. Glanville, Lessons learned during the successful eradication of bovine tuberculosis from Australia. *Vet. Rec.* **177**, 224–232 (2015). doi: 10.1136/vr.103163; pmid: 26338937
3. A. L. Olmstead, P. W. Rhode, An Impossible Undertaking: The Eradication of Bovine Tuberculosis in the United States. *J. Econ. Hist.* **64**, 734–772 (2004). doi: 10.1017/S0022050704002955
4. J. McCormack, Horizon scanning: What next for bovine TB control in England? *Ir. Vet. J.* **76** (Suppl 1), 18 (2023). doi: 10.1186/s13620-023-00242-z; pmid: 37525221
5. S. J. More, bTB eradication in Ireland: Where to from here? *Ir. Vet. J.* **76**, 11 (2023). doi: 10.1186/s13620-023-00239-8; pmid: 37403121
6. P. G. Livingstone, N. Hancox, G. Nugent, G. W. de Lisle, Toward eradication: The effect of Mycobacterium bovis infection in wildlife on the evolution and future direction of bovine tuberculosis management in New Zealand. *N. Z. Vet. J.* **63** (Suppl 1), 4–18 (2015). doi: 10.1080/00480169.2014.971082; pmid: 25273888
7. A. J. Conlan *et al*., Potential benefits of cattle vaccination as a supplementary control for bovine tuberculosis. *PLOS Comput. Biol.* **11**, e1004038 (2015). doi: 10.1371/journal.pcbi.1004038; pmid: 25695736
8. EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), Scientific Opinion on field trials for bovine Tuberculosis vaccination. *EFSA J.* **11**, 3475 (2013). doi: 10.2903/j.efsa.2013.3475
9. A. O. Whelan *et al*., Lack of correlation between BCG-induced tuberculin skin test sensitisation and protective immunity in cattle. *Vaccine* **29**, 5453–5458 (2011). doi: 10.1016/j.vaccine.2011.05.057; pmid: 21640776
10. A. O. Whelan *et al*., Development of a skin test for bovine tuberculosis for differentiating infected from vaccinated animals. *J. Clin. Microbiol.* **48**, 3176–3181 (2010). doi: 10.1128/JCM.00420-10; pmid: 20592155
11. B. Bayissa *et al*., Field evaluation of specific mycobacterial protein-based skin test for the differentiation of Mycobacterium bovis-infected and Bacillus Calmette Guerin-vaccinated crossbred cattle in Ethiopia. *Transbound. Emerg. Dis.* **69**, e1–e9 (2022). doi: 10.1111/tbed.14252; pmid: 34331511
12. S. Srinivasan *et al*., A defined antigen skin test for the diagnosis of bovine tuberculosis. *Sci. Adv.* **5**, eaax4899 (2019). doi: 10.1126/sciadv.aax4899; pmid: 31328169
13. S. Srinivasan *et al*., A Defined Antigen Skin Test That Enables Implementation of BCG Vaccination for Control of Bovine Tuberculosis: Proof of Concept. *Front. Vet. Sci.* **7**, 391 (2020). doi: 10.3389/fvets.2020.00391; pmid: 32793643
14. R. Firdessa *et al*., High prevalence of bovine tuberculosis in dairy cattle in central ethiopia: Implications for the dairy industry and public health. *PLOS ONE* **7**, e52851 (2012). doi: 10.1371/journal.pone.0052851; pmid: 23285202
15. G. A. Mekonnen *et al*., Prevalence of bovine tuberculosis and its associated risk factors in the emerging dairy belts of regional cities in Ethiopia. *Prev. Vet. Med.* **168**, 81–89 (2019). doi: 10.1016/j.prevetmed.2019.04.010; pmid: 31097127
16. G. Almaw *et al*., The variable prevalence of bovine tuberculosis among dairy herds in Central Ethiopia provides opportunities for targeted intervention. *PLOS ONE* **16**, e0254091 (2021). doi: 10.1371/journal.pone.0254091; pmid: 34214106
17. S. Srinivasan *et al*., Prevalence of Bovine Tuberculosis in India: A systematic review and meta-analysis. *Transbound. Emerg. Dis.* **65**, 1627–1640 (2018). doi: 10.1111/tbed.12915; pmid: 29885021
18. B. Sibhat *et al*., Bovine tuberculosis in Ethiopia: A systematic review and meta-analysis. *Prev. Vet. Med.* **147** (Supplement C), 149–157 (2017). doi: 10.1016/j.prevetmed.2017.09.006; pmid: 29254713
19. S. Srinivasan *et al*., A Meta-Analysis of the Effect of Bacillus Calmette-Guérin Vaccination Against Bovine Tuberculosis: Is Perfect the Enemy of Good? *Front. Vet. Sci.* **8**, 637580 (2021). doi: 10.3389/fvets.2021.637580; pmid: 33681334
20. P. Mangtani *et al*., Protection by BCG vaccine against tuberculosis: A systematic review of randomized controlled trials. *Clin. Infect. Dis.* **58**, 470–480 (2014). doi: 10.1093/cid/cit790; pmid: 24336911
21. E. Shim, A. P. Galvani, Distinguishing vaccine efficacy and effectiveness. *Vaccine* **30**, 6700–6705 (2012). doi: 10.1016/j.vaccine.2012.08.045; pmid: 22944629
22. G. Lopez-Valencia *et al*., Field evaluation of the protective efficacy of Mycobacterium bovis BCG vaccine against bovine tuberculosis. *Res. Vet. Sci.* **88**, 44–49 (2010). doi: 10.1016/j.rvsc.2009.05.022; pmid: 19564029
23. M. C. M. De Jong, T. G. Kimman, Experimental quantification of vaccine-induced reduction in virus transmission. *Vaccine* **12**, 761–766 (1994). doi: 10.1016/0264-410X(94)90229-1; pmid: 8091855
24. K. Orsel, M. C. M. de Jong, A. Bouma, J. A. Stegeman, A. Dekker, Foot and mouth disease virus transmission among vaccinated pigs after exposure to virus shedding pigs. *Vaccine* **25**, 6381–6391 (2007). doi: 10.1016/j.vaccine.2007.06.010; pmid: 17658199
25. A. J. K. Conlan, M. Vordermeier, M. C. de Jong, J. L. Wood, The intractable challenge of evaluating cattle vaccination as a control for bovine Tuberculosis. *eLife* **7**, e27694 (2018). doi: 10.7554/eLife.27694; pmid: 29866255
26. A. G. J. Velthuis, M. C. M. De Jong, E. M. Kamp, N. Stockhofe, J. H. M. Verheijden, Design and analysis of an Actinobacillus pleuropneumoniae transmission experiment. *Prev. Vet. Med.* **60**, 53–68 (2003). doi: 10.1016/S0167-5877(03)00082-5; pmid: 12900149
27. A. G. J. Velthuis, A. Bouma, W. E. A. Katsma, G. Nodelijk, M. C. M. De Jong, Design and analysis of small-scale transmission experiments with animals. *Epidemiol. Infect.* **135**, 202–217 (2007). doi: 10.1017/S095026880600673X; pmid: 17291360
28. H. M. Vordermeier, G. J. Jones, B. M. Buddle, R. G. Hewinson, B. Villarreal-Ramos, Bovine Tuberculosis in Cattle: Vaccines, DIVA Tests, and Host Biomarker Discovery. *Annu. Rev. Anim. Biosci.* **4**, 87–109 (2016). doi: 10.1146/annurev-animal-021815-111311; pmid: 26884103
29. G. A. Mekonnen, G. Ameni, J. L. N. Wood, S. Berg, A. J. K. Conlan; ETHICOBOTS consortium, Network analysis of dairy cattle movement and associations with bovine tuberculosis spread and control in emerging dairy belts of Ethiopia. *BMC Vet. Res.* **15**, 262 (2019). doi: 10.1186/s12917-019-1962-1; pmid: 31349832
30. G. Meisinger, Economic effects of the elimination of bovine tuberculosis on the productivity of cattle herds. 2. Effect on meat production. *Monatsh. Veterinarmed.* **25**, 7–13 (1970). pmid: 5519247
31. I. Aznar *et al*., Quantification of Mycobacterium bovis transmission in a badger vaccine field trial. *Prev. Vet. Med.* **149**, 29–37 (2018). doi: 10.1016/j.prevetmed.2017.10.010; pmid: 29290298
32. S. P. Carter *et al*., BCG vaccination reduces risk of tuberculosis infection in vaccinated badgers and unvaccinated badger cubs. *PLOS ONE* **7**, e49833 (2012). doi: 10.1371/journal.pone.0049833; pmid: 23251352
33. N. A. Parlane *et al*., Revaccination of cattle with bacille Calmette-Guérin two years after first vaccination when immunity has waned, boosted protection against challenge with Mycobacterium bovis. *PLOS ONE* **9**, e106519 (2014). doi: 10.1371/journal.pone.0106519; pmid: 25180583
34. H. Lemma, K. Belihu, D. Sheferaw, Study on the reproductive performance of Jersey cows at Wolaita Sodo dairy farm, Southern Ethiopia. *Ethiop. Vet. J.* **14**, 53–70 (2010).
35. E. Brooks-Pollock, J. L. N. Wood, Eliminating bovine tuberculosis in cattle and badgers: Insight from a dynamic model. *Proc. Biol. Sci.* **282**, 20150374 (2015). doi: 10.1098/rspb.2015.0374; pmid: 25972466
36. A. G. J. Velthuis, M. C. M. De Jong, J. De Bree, Comparing methods to quantify experimental transmission of infectious agents. *Math. Biosci.* **210**, 157–176 (2007). doi: 10.1016/j.mbs.2007.04.009; pmid: 17604060
37. B. Goodrich, J. Gabry, I. Ali, S. Brilleman, rstanarm: Bayesian applied regression modeling via Stan. (R package version 2.21.3, 2022); https://mc-stan.org/rstanarm.
38. R Core Team, R: A language and environment for statistical computing (R Foundation for Statistical Computing, 2019); https://www.R-project.org/.
39. S. Widgren, P. Bauer, R. Eriksson, S. Engblom, SimInf: An R Package for Data-Driven Stochastic Disease Spread Simulations. *J. Stat. Softw.* **91**, 1–42 (2019). doi: 10.18637/jss.v091.i12
40. K. Willgert, A. J. K. Conlan, MonkeyMyshkin/BCGCrossover: BCG vaccination of cattle reduces transmission of bovine tuberculosis, improving the prospect of elimination, version 0.1.4, Zenodo (*2024*); https://zenodo.org/doi/10.5281/zenodo.10417489.
41. L. J. S. Allen, "An introduction to stochastic epidemic models" in *Mathematical Epidemiology*, F. Brauer, P. van den Driessche, J. Wu, Eds. (Springer, 2008), vol. 1945 of *Lecture Notes in Mathematics*, pp. 81–130. doi: 10.1007/978-3-540-78911-6_3
42. S. L. Hui, S. D. Walter, Estimating the error rates of diagnostic tests. *Biometrics* **36**, 167–171 (1980). doi: 10.2307/2530508; pmid: 7370371
43. J. Collins, M. Huynh, Estimation of diagnostic test accuracy without full verification: A review of latent class methods. *Stat. Med.* **33**, 4141–4169 (2014). doi: 10.1002/sim.6218; pmid: 24910172
44. M. Schweinberger, P. N. Krivitsky, C. T. Butts, J. Stewart, Exponential-Family Models of Random Graphs: Inference in Finite-, Super-, and Infinite Population Scenarios. *Stat. Sci.* **35**, 627–662 (2020). doi: 10.1214/19-STS743

45. P. N. Krivitsky, D. R. Hunter, M. Morris, C. Klumb, ergm 4: New features for Analyzing Exponential-Family Random Graph Models. *J. Stat. Softw.* **105**, 1–44 (2023). doi: 10.18637/jss.v105.i06
46. H. Vordermeier, B. Sidders, N. Stoker, K. Ewer, Mycobacterium antigens. WIPO Patent WO/2009/060184 (2009).
47. H. Vordermeier, A. Whelan, Diagnostic reagents. WIPO Patent WO/2011/135369 (2011).
48. G. Jones, H. Vordermeier, Diagnostic reagents. WIPO Patent WO/2012/010875 (2012).
49. V. Kapur, S. Srinivasan, H. Vordermeier, G. Jones, Diagnostic reagents. WIPO Patent WO/2020/208368 (2020).

ACKNOWLEDGMENTS

We gratefully acknowledge support from members of the ETHICOBOTS consortium, including A. Aseffa, A. Mihret, B. Tessema, B. Belachew, E. Fekadu, F. Melese, G. Gemechu, H. Taye, R. Tschopp, S. Haile, S. Ayalew, and T. Hailu at the Armauer Hansen Research Institute, Ethiopia; R. Tschopp at the Swiss Tropical and Public Health Institute, Switzerland; A. Bekele, C. Yirga, M. Ambaw, T. Mamo, and T. Solomon at the Ethiopian Institute of Agricultural Research, Ethiopia; T. Teklewold at the Amhara Regional Agricultural Research Institute, Ethiopia; S.G., G. Gari, M. Sahle, A. Aliy, A. Olani, A.S., G. Almaw, G.M., M. Tamiru, and S. Guta at the National Animal Health Diagnostic and Investigation Center, Ethiopia; J.W. (consortium lead author), A.J.K.C., and A. Clarke at Cambridge University, UK; H. L. Moore and C. Hodge at University College London, UK; C. Smith at University of Manchester, UK; R. Glyn Hewinson, S.B., H.M.V., and J. Nunez-Garcia at the Animal and Plant Health Agency, UK; and G.A., B.B., A. Zewude, A. Worku, L. Terfassa, M. Chanyalew, T. Mohammed, and M. Zeleke at Addis Ababa University, Ethiopia. We also thank members of the ABTBC consortium (listed alphabetically by institution): A.F., B.G., B.B., D. Worku, G. Bahiru, G.A., M.G., and Y. Zeleke (Addis Ababa University, Ethiopia); G. Jones, H.M.V., P. Hogarth, and S.B. (the Animal and Plant Agency, UK); A.S., B.T., M.L., S.G., T. Rufael, and W.B. (the Animal Health Institute, Ethiopia); A. Mihret (Armaeur Hansen Research Institute, Ethiopia); A.J.K.C., K.W., and J.W. (Cambridge University, UK); M.V. (CisGen Biotech Discoveries, India); P. Dandapat (Indian Veterinary Research Institute, India); N. Jindal (Lala Lajpat Rai University of Veterinary and Animal Sciences, India); D.B. (Lelystad, Netherlands); M. Nagalingam (National Institute of Veterinary Epidemiology and Disease Investigation, India); M. K. Papanna, R. Katani, V. Thapa, and V.K. (lead consortium author) (The Pennsylvania State University, USA); G. Hewinson (University of Wales, UK); and C. Costanzo, H. Martinez, J. Lombard, and T. Thacker (US Department of Agriculture, Animal and Plant Health Inspection Services). **Funding:** This research was supported by the Bill & Melinda Gates Foundation (in partnership with the UK Foreign, Commonwealth and Development Office), grant OPP1176950; the Biotechnology and Biological Sciences Research Council (BBSRC), the Department for International Development, the Economic and Social Research Council, the Medical Research Council, the Natural Environment Research Council, and the Defense Science and Technology Laboratory under the Zoonoses and Emerging Livestock Systems (ZELS) program, grant BB/L018977/1; and by BBSRC grant BB/M011194/1 to K.W. **Ethical approval:** The study was approved and granted ethical clearance by the Institutional Review Board of Aklilu Lemma Institute of Pathobiology, reference number ALIPB IRB/44/2013/21. All relevant local biosecurity and safety procedures pertaining to animals were adhered to. **Author contributions:** Conceptualization: A.J.K.C., D.B., G.A., J.W., M.C.M.d.J., N.J., and V.K. Methodology: A.J.K.C., D.B., G.A., H.M.V., S.B., J.W., K.W., M.C.M.d.J., M.V., N.J., S.S., and V.K. Investigation: A.F., A.J.K.C., A.S., B.B., B.G., B.T., G.M., K.W., M.G., M.L., S.G., S.S., T.R., and W.B. Visualization: A.J.K.C., K.W., and V.K. Funding acquisition: A.J.K.C., D.B., G.A., H.M.V., J.W., K.W., M.V., and V.K. Project administration: V.K. Supervision: A.J.K.C., B.G., G.A., S.B., and V.K. Writing – original draft: A.F., A.J.K.C., K.W., and V.K. Writing – review and editing: All authors. **Competing interests:** APHA (the former employer for H.M.V.) holds three patents [patent numbers WO/2009/060184 (*46*), WO/2011/135369 (*47*), and WO/2012/010875 (*48*)] for the use of Rv3615c in diagnostic tests for bTB. In addition, APHA and Penn State (S.S. and V.K.) are in the process of intellectual property protection filings for a peptide-based DIVA skin test [WO/2020/208368 (*49*)]. S.S. is also affiliated with the Department of Epidemiology and Biostatistics, College of Human Medicine, Michigan State University, East Lansing, MI, USA. The authors declare that they have no other competing interests. **Data and materials availability:** All data and code are available in the main text or the supplementary materials and at GitHub (https://github.com/MonkeyMyshkin/BCGCrossover) and Zenodo (*40*). This research was funded in whole or in part by the Bill & Melinda Gates Foundation (OPP1176950) and the Biotechnology and Biological Sciences Research Council (BBSRC) (BB/M011194/1 and BB/L018977/1), cOAlition S organizations. The author will make the Author Accepted Manuscript (AAM) version available under a CC BY public copyright license.

SUPPLEMENTARY MATERIALS

Submitted 16 October 2023; accepted 24 January 2024
10.1126/science.adl3962