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Science & Society

The Tick Project: Testing Environmental Methods of Preventing Tick-borne Diseases

Felicia Keesing[1,*] and Richard S. Ostfeld[2]

Prevention of tick-borne diseases in humans is challenging. To date, no prevention strategies have been shown to be consistently effective. Here, we describe the design of a new large-scale study, involving hundreds of households in Dutchess County, New York, testing whether environmental interventions, applied intensively and over 4 years, can prevent human cases.

Worldwide, tick-borne diseases afflict hundreds of thousands of people every year, with many of those cases occurring in the USA [1,2]. The most common tick-borne illness in the USA, by far, is Lyme disease, which is caused by infection with *Borrelia burgdorferi*, a spirochete bacterium [2]. A number of other tick-borne diseases are expanding rapidly. Among these are other bacterial diseases, such as anaplasmosis and Rocky Mountain spotted fever, as well as diseases caused by protozoans and viruses, including babesiosis and Powassan virus encephalitis [3]. Understandably, considerable attention is focused on accurate diagnosis and effective treatment of these infections. Prevention of exposure to these infections has received far less attention from the scientific and funding communities.

Preventing tick-borne diseases is a challenge. Over the last century, vaccines for infectious diseases have prevented millions of illnesses and deaths from diseases ranging from measles to rabies. But there is not a single human vaccine on the market for even one tick-borne disease in the USA. Even if there were a vaccine for, say, Lyme disease, people living in Lyme disease-endemic areas would still need to remain vigilant about exposures to ticks because of the abundance of other tick-borne infections. To counter this problem, some researchers are trying to develop anti-tick vaccines [4,5], though such a product is likely years from comprehensive testing and commercial release.

At present, the best alternative for preventing tick-borne diseases is to reduce people's exposure to infected ticks. This could be achieved by reducing overall tick abundance, by reducing the proportion of ticks that are infected, by changing human behaviors that affect encounters with ticks, or by a combination of all three. Unfortunately, though many prevention strategies have been assessed, few have addressed human outcomes, and none of these have been found to be consistently effective [6]. For example, Hinckley *et al*. [7] conducted a large-scale experiment to test whether bifenthrin, an acaricide, used on perimeters of people's yards reduced the incidence of Lyme disease. Working with thousands of participants, they found that the pesticide treatment reduced tick abundance in treated areas by 63%, but that there was no corresponding reduction in the number of human cases [7].

Why might reducing tick abundance by 63% in people's yards fail to reduce the number of human cases? Reducing the incidence of human cases might require an even greater reduction in tick abundance, or the treatment of entire yards rather than just edges. People might also be more likely to be exposed to infected ticks when they are outside of their yards, for example in a park or elsewhere in their neighborhood. In the absence of a method demonstrated to reduce cases strongly and consistently, residents of tick-prone areas are left with an array of unproven options.

We established the Tick Project in 2016 to test whether environmental interventions could prevent cases of tick-borne diseases. Because no prior efforts have demonstrated an effect on human cases, we increased the intensity and spatial scale of our design compared to past efforts in order to determine whether an environmental approach could work under the best circumstances. Building on the results of the Hinckley *et al*. study [7], we chose to test two complementary methods of tick reduction rather than just one, and we chose to treat multiproperty neighborhoods rather than individual properties. We also chose to treat entire properties, not just perimeters.

The project is taking place in Dutchess County, New York, an area of high incidence for tick-borne diseases. Our thousands of participants live in 24 neighborhoods, each of which has a high incidence of tick-borne diseases. To enroll people in the study, we began recruiting participants from candidate neighborhoods in 2016. Using phone-number look-up services and door-to-door canvasing, we contacted residents living within the boundaries of target neighborhoods and invited them to participate. Each neighborhood consists of about 100 households (Figure 1A), and has at least 25% of its households enrolled (Figure 2B). Enrolling and managing thousands of participants required the development of a sophisticated method for tracking participants, communications, and documents. We worked with developers at Arkus, Inc. to convert Salesforce, a commercial software application, from a corporate-focused client

Figure 1. (A) The Tick Project Is Being Conducted in 24 Neighborhoods, Each of Which Consists of ~100 Properties. In each participating neighborhood, at least 35% of households are enrolled. (B) Each property is treated with *Met52* fungal spray and Tick Control System rodent bait boxes with either active or inactive (placebo) ingredients. *Met52* is applied to the entire yard except for impervious surfaces; bait boxes (*) are distributed across the property, as indicated for this representative property. Images from Google Maps. (Purchased from Shutterstock).

relationship manager to a research-focused participant relationship manager. The proprietary software that resulted from these partnerships has many benefits for a project of this size, including the capacity to use sophisticated mass-marketing tools to communicate with our participants.

Once we had enrolled enough participants, we randomly assigned each neighborhood to one of four combinations of two treatments (Figure 2A), both of which are designed to kill ticks. The first treatment is a commercial spray, *Met52* (Novozymes Biologicals, Inc., Salem, VA), made from the spores of a native entomopathogenic fungus, *Metarhizium brunneum* (=*M. anisopliae*) that has been shown to kill ticks [8]. The second treatment is called the Tick Control System (Select TCS; Tick Box Technology Corporation, Norwalk, CT), and consists of a small metal-covered box containing bait that attracts small mammals, including rodents. Once inside, animals are treated with a dab of an acaricide – fipronil – that kills ticks on animals for several weeks (Figure 1B). The study is randomized and replicated with six neighborhoods treated with each of the four treatment combinations (Figure 2A). The study is also placebo-controlled; all of the neighborhoods receive applications of both of our interventions, but some of the interventions are placebos. The placebo version of the *Met52* fungal spray is water, while the placebo TCS boxes contain no fipronil. The study is also double-blind – neither the participants nor the field personnel working on the properties know which treatment combination each neighborhood is receiving.

Our choice of which treatments to use in this experiment was guided by three criteria. First, we required that treatments had already been shown to be effective at reducing tick abundance under realistic conditions. Second, we required products to be commercially available so that there would be no delay in the ability of communities to implement the treatments if they were found to be effective. Finally, we required that our treatments had to be safe for people, pets, and the environment. This ruled out some products, like bifenthrin, which can have substantial nontarget effects. The active ingredient in our *Met52* treatment is particularly effective at killing ticks but can also be lethal to some other arthropods. In 2016, we conducted a field study to determine potential effects of *Met52* on nontarget arthropods in soil and leaf litter and found no significant effects [9]. Our second treatment, the TCS bait box, targets ticks on small mammals, which are responsible for infecting the majority of ticks with tick-borne pathogens in the northeastern USA. This product delivers a minute volume of fipronil to small mammals and does not involve environmental release; there are no known negative effects on people, pets, or the environment.

Our response variables following these treatments fall into three categories. First, we collect data on risk factors for tick-borne diseases, including the abundance and infection prevalence of ticks in yards and the number of ticks on rodent hosts [10]. We also use wildlife cameras to monitor the composition of host communities in each neighborhood [11]. Second, we monitor tick encounters and cases of tick-borne diseases in study participants. Every 2 weeks, between April and December, we survey a contact person in each household to determine whether anyone in the household (including a dog or a cat) has encountered a tick or been diagnosed with a tick-borne disease. Messages requesting this information are sent via SMS or email depending on preferences established by the participant. A small percentage of participants require

(A)

	Met52 fungal spray	Placebo fungal spray
Select TCS bait boxes	6 neighborhoods	6 neighborhoods
Placebo bait boxes	6 neighborhoods	6 neighborhoods

(B)

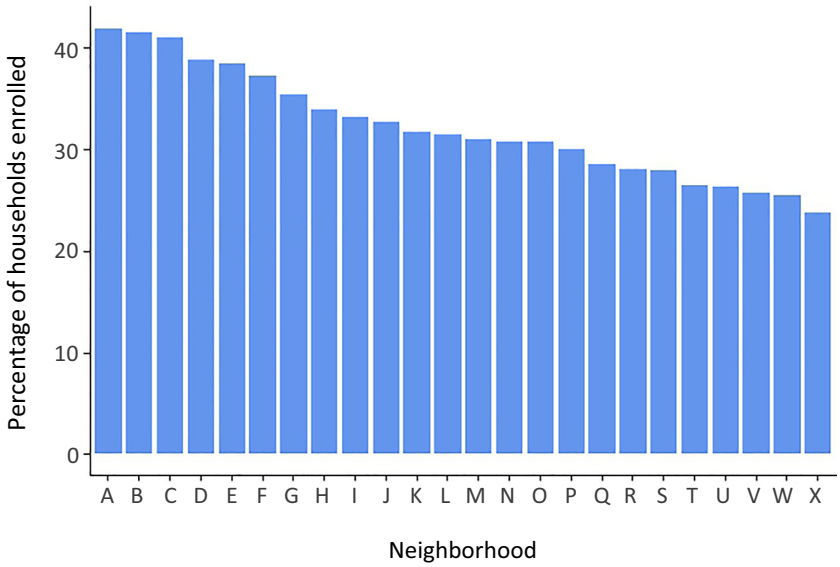

Figure 2. (A) Experimental Design of the Tick Project. Each treatment combination is being tested in six neighborhoods, and each treatment has a corresponding placebo control. The assignment of treatment and control conditions to neighborhoods is double-blind – neither the participants nor those collecting data for the study know which treatments are being applied to which neighborhoods. (B) Enrollment levels in the Tick Project, showing the percentage of households participating in each of the 24 neighborhoods.

telephone contacts. If participants respond that they have had a tick-borne illness or encountered a tick, we ask them to complete questions on a more detailed electronic survey. The flow of information in our mass communications with participants is managed by our software. Finally, we confirm self-reported cases of tick-borne illness of humans by contacting participants' health care providers after receiving appropriate permissions from participants. We hypothesize that the number of ticks per yard and the number of human cases will be lower in neighborhoods receiving treatments that include the active ingredients.

We began applying treatments to properties in spring 2017, and treatments will continue through 2020, so that we will have 4 years of data on these neighborhoods. The long-term nature of the study is based on the expectation that the effects of the treatments will be cumulative. For instance, by killing immature ticks attached to small mammals, the TCS bait boxes should affect host-seeking ticks (the stages capable of transmitting infection to people) the following year. And, any reductions in the abundance of host-seeking ticks should reduce both tick-to-host and host-to-tick transmission rates of pathogens, leading to a positive feedback that should further reduce the abundance of infected ticks and the number of cases.

We expect that a 4-year, randomized, placebo-controlled, double-blind design, with two tick-control methods and a relatively high level of replication, conducted at the level of residential neighborhoods, will answer the question of whether an intensive community-based strategy can prevent tick-borne infections. If it can, of course many important questions will remain, including what the minimum level of participation is and who should bear the cost of treating high-risk neighborhoods.

Acknowledgments

The Tick Project is supported by a grant from the Steven and Alexandra Cohen Foundation, with additional financial and in-kind support from the Centers for Disease Control, the New York State Legislature, the Dutchess County Water and Wastewater Authority, and a number of private donors. We thank our partners, the Cary Institute of Ecosystem Studies, Bard College, the Centers for Disease Control, the New York State Department of Health, and the Dutchess County Department of Community and Behavioral Health. The Cary Institute of Ecosystem Studies provides extensive logistical support. We have benefited enormously from the expertise and dedication of the team at Arkus, Inc., especially Amy Buccifero. Salesforce.org, Pardot, SMSMagic, FormAssembly, and DocUSign provide software and support at reduced fees. Our colleagues at Pestech Pest Solutions provide the professional applications of our treatments. We are deeply indebted to the people who make the project work every day: Deanna DePietro, Shannon Duerr, Ilya Fischhoff, Stacy Mowry, Jennifer Pendleton, Ashley Pfister, and Alex Wolf. Our field crews are indispensable – thanks to Daniela Azulai, Katelyn BaDour, Dylan Dahan,

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Michelle Ferrell, Sara McBride, Tori Palfini, Luke Porter, Sophia Raithel, Katie Sweeney, Marissa Teator, Makayla Syas, Charlene Gray Tarsa, Alison Molnar, Anna Butler, Beckett Lansbury, Megan Schierer, Mica McCarty-Glenn, Nadine Pershyn, Nicholas Petterelli, Nicole Pierro, Samantha Sambado, Shefali Azad, Vince Meyer, Mohammad Bayan Harris, and Rachel Livengood. The project has benefited enormously from the professionalism and support of Josh Ginsberg, Holly Talbot, Lori Quillen, David Fischer, Amanda Johnson, Rob Robinson, Alison Hinckley, Ben Beard, Lars Eisen, Paul Mead, Sarah Hook, Jill Auerbach, Sue Serino, and Sarah Dunphy-Lelii. This research would not be possible without the enthusiastic participation of thousands of residents of Dutchess County, New York. Three anonymous reviewers provided valuable and constructive comments on an earlier version of the manuscript.

[1]Program in Biology, Bard College, Annandale, NY 12504, USA
[2]Cary Institute of Ecosystem Studies, Millbrook, NY 12545, USA

*Correspondence: keesing@bard.edu (F. Keesing).

https://doi.org/10.1016/j.pt.2018.02.008

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Spotlight

Sex in *Plasmodium falciparum:* Silence Play between GDV1 and HP1

Edward Rea,[1] Karine G. Le Roch,[2] and Rita Tewari[1,*]

Understanding how malaria parasites commit to sexual development is key to the development of transmission-blocking strategies. Recent work by Filarsky and colleagues extends our understanding of the molecular mechanisms driving this process by characterizing an early factor in gametocytogenesis, and showing how this fits neatly into our current knowledge of sexual commitment.

Malaria, caused by apicomplexan parasites of the *Plasmodium* genus, is a vector-borne disease that affects 216 million people and leads to approximately 445 000 deaths annually, as reported by WHO in 2017. Whilst the rapid asexual proliferation of malaria parasites within the erythrocytes of vertebrate hosts leads to the pathology and clinical symptoms of the disease, it is the sexual stages that are responsible for the transmission between vertebrate host and mosquito vector and the spread of the parasite. Hence, understanding the molecular mechanisms controlling gametocytogenesis, the formation and maturation of male and female gametocytes in the vertebrate bloodstream, will be essential if we want to develop novel intervention strategies to eradicate the disease.

Until recently little was known of the molecules that trigger or regulate sexual commitment in the parasite life cycle. In 2012, Eksi *et al*. showed that gametocyte development 1 (GDV1), a perinuclear *Plasmodium falciparum* protein, plays a role in gametocytogenesis [1]. GDV1 overexpression enhanced gametocyte formation, whereas *gdv1*-deleted parasite lines were gametocyte deficient. Further seminal papers in both *P. falciparum* and *Plasmodium berghei* identified the master regulator of gametocytogenesis: AP2-G, a member of the apiAP2 transcription factor family that was found to control the switch between asexual and sexual development [2,3]. Additional studies identified heterochromatin protein 1 (HP1) and histone deacetylase 2 (HDA2) as epigenetic regulators of sexual commitment that control AP2-G expression, strongly suggesting that gametocytogenesis is under epigenetic control [4,5]. Despite these discoveries, the function of GDV1, and how it works in concert with HP1, remained unclear.

In a new study in *Science*, Filarsky *et al*. [6] dissected the function of GDV1 in *P. falciparum* using elegant and complementary methodologies, including conditional CRISPR-Cas9 gene editing and ChIP-seq experiments. The authors showed that GDV1 evicts HP1 from H3K9me3 sites in the parasite genome, thereby depressing AP2-G expression and inducing gametocytogenesis. Intriguingly, they provide evidence that GDV1 expression is controlled by a GDV1 antisense RNA, and that GDV1 protein acts by antagonising epigenetic silencing of HP1 (Figure 1).

Filarsky *et al*. [6] show that GDV1 binds HP1, both *in vivo* and *in vitro*, through reciprocal immunoprecipitation and cellular colocalization studies, suggesting that they form a regulatory complex that functions to activate gametocytogenesis. Using an FKBP/Shield-1 conditional expression system, the authors revealed