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Long-Term Effects of Tetanus Toxoid Inoculation on the Demography and Life Expectancy of the Cayo Santiago Rhesus Macaques

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Abstract

Tetanus was a major cause of mortality in the free-ranging population of rhesus monkeys on Cayo Santiago prior to 1985 when the entire colony was given its first dose of tetanus toxoid. The immediate reduction in mortality that followed tetanus toxoid inoculation (TTI) has been documented, but the long-term demographic effects of eliminating tetanus infections have not. This study uses the Cayo Santiago demographic database to construct comparative life tables 12 years before, and 12 years after, TTI. Life tables and matrix projection models are used to test for differences in: (i) survival among all individuals as well as among social groups, (ii) long-term fitness of the population, (iii) age distribution, (iv) reproductive value, and (v) life expectancy. A retrospective life table response experiment (LTRE) was performed to determine which life cycle transition contributed most to observed changes in long-term fitness of the population post-TTI. Elimination of clinical tetanus infections through mass inoculation improved the health and well-being of the monkeys. It also profoundly affected the population by increasing survivorship and long-term fitness, decreasing the differences in survival rates among social groups, shifting the population's age distribution towards older individuals, and increasing reproductive value and life expectancy. These findings are significant because they demonstrate the long-term effects of eradicating a major cause of mortality at a single point in time on survival, reproduction, and overall demography of a naturalistic population of primates.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article.

Keywords

Macaca mulatta; matrix projection model; life table; life table response experiment; survivorship; mortality

INTRODUCTION

Tetanus, also known as “lockjaw,” is a severe disease of the central nervous system, primarily of primates, horses, and other farm animals caused by the potent neurotoxin, tetanospasmin, produced by the gram-positive, spore-forming anaerobic bacteria, *Clostridium tetani*, at the site of necrotic infections and puncture wounds [Wassilak & Kretsinger, 2009]. The organism is commonly found in animal feces and soil [Gillespie & Timoney, 1988]. The diagnosis of tetanus in humans and animals is presumptive and based primarily on clinical signs, wounding and vaccination history because the organism is difficult to culture from the site of infection and because it can be isolated from patients who do not have tetanus [Wassilak & Kretsinger, 2009]. *Clostridium tetani* can also be detected in wounds using PCR [Nakano et al., 2012] but, like culturing, this does not necessarily indicate cause and effect. Serological tests are of little value in diagnosing tetanus as the amount of tetanus toxin necessary to cause disease is insufficient for the body’s immune system to produce antitoxin [Wassilak & Kretsinger, 2009]. Definitive diagnosis depends on the demonstration of antitoxin production in mice using the mouse toxin neutralization test [Hardegree et al., 1982; Kessler & Rawlins, 1984; Wassilak & Kretsinger, 2009]. However, it is widely accepted that there is no natural immunity to *Clostridium tetani* and tetanus antitoxin does not develop from infection. Second cases of tetanus have been reported in the same humans and macaques [DiGiacomo & Missakian, 1972; Hill, 1936; Kessler & Rawlins, 1984; Wassilak & Kretsinger, 2009]. There are no pathognomic gross or histological lesions of tetanus although necropsy findings almost always include lock jaw and extensor rigor, and necrotic wounds can be found in many cases [DiGiacomo & Missakian, 1972; Rawlins & Kessler, 1982; Springer et al., 2009; Wassilak & Kretsinger, 2009].

The first detailed description of tetanus in nonhuman primates was in Sri Lankan toque macaques (*Macaca sinica*), mostly belted pets [Hill, 1936]. The signs and symptoms included most of those reported for humans: reluctance to move or eat, progressive stiffness and adduction of the pectoral limbs, piloerection, trismus (lock jaw) and *risus sardonicus*, extensor rigidity, opisthotonus, status epilepticus, respiratory failure, and cardiac arrest. Hill [1936] also observed bipedal locomotion and hopping. The classical human triad of trismus (lock jaw and *risus sardonicus*), extensor rigidity and opisthotonus, along with the other signs reported by Hill [1936] are similar to those reported in other macaques, baboons and squirrel monkeys [DiGiacomo & Missakian, 1972; Goodwin et al., 1987; Kessler & Brown, 1979; Nakano et al., 2012; Rawlins & Kessler, 1982; Springer et al., 2009]. Unlike many other diseases of the central nervous system, victims of tetanus remain conscious until death [Hill, 1936; Rawlins & Kessler, 1982; Wassilak & Kretsinger, 2009].

Tetanus was first recognized in the Cayo Santiago rhesus macaques in 1939, shortly after their release onto the island [Sade et al., 1985] and mentioned in an article about the colony in 1954 [Litter, 1954]. However, it was not until 1972 that a detailed report on tetanus in the population was published [DiGiacomo & Missakian, 1972]. Subsequent studies found that tetanus was a major cause of mortality in the population [Kessler et al., 1988; Rawlins & Kessler, 1982]. Using a conservative case definition, tetanus accounted for 19.5% to 24.7% of all deaths from 1976 through 1984 [Kessler et al., 1988; Rawlins & Kessler, 1982]. There was no significant annual variation in either the total or tetanus mortality rates, and although the population increased from 479 to 914 monkeys from 1976 to 1981, there was no evidence for a density-dependent increase in either the total or tetanus mortality rates [Rawlins & Kessler, 1982]. Most tetanus cases occurred during the mating seasons from contaminated fight wounds but post-partum and neonatal cases were also observed [Rawlins & Kessler, 1982]. Although Hill [1936] was successful in saving three of five tetanic toque macaques by administering intra-theal tetanus antitoxin and some rhesus have been recovered using intravenous tetanus antitoxin combined with intramuscular tetanus toxoid and high doses of systemic antibiotics, the case fatality rate in the Cayo Santiago rhesus macaques ranged from 83% to 85% [DiGiacomo & Missakian, 1972; Rawlins & Kessler, 1982] and in some species, such as squirrel monkeys, can reach 100% despite treatment [Kessler & Brown, 1979; Kessler & Martinez, 1979; Springer et al., 2009].

Tetanus is completely preventable in humans using prophylactic tetanus toxoid [Wassilak & Kretsinger, 2009]. Evidence that tetanus toxoid inoculation (TTI) in the Cayo Santiago rhesus population might be effective came from a blind experiment conducted in December 1973 [Sade et al., 1985]. Half ($n = 23$) of the birth crop was randomly inoculated with a single dose of tetanus toxoid. Four years later significant differences in survivorship between the control and TTI groups were found [Sade et al., 1985]. The effectiveness of this pilot project in reducing mortality was later confirmed over a longer period by following up on the survivors of the original 47 animals [Kessler et al., 1988]. Over 11 years (1973–1983), 41.7% of the control group and only 17.2% of the TTI monkeys died. Thus, monkeys protected from tetanus infections had a mortality rate that was 58.3% lower than uninoculated animals. This significant difference was attributed to tetanus prevention through TTI as no management changes were made in the colony [Kessler et al., 1988, Kessler et al., 2006].

Due to the lack of natural immunity to tetanus and the serious health threat caused by tetanus to the monkeys on Cayo Santiago [Kessler & Rawlins, 1984], the decision was made to inoculate the entire population with Supertet[®] starting in 1985 [Kessler et al., 1988; Kessler et al., 2006]. Total deaths and mortality rates in the colony decreased significantly after TTI and tetanus deaths plummeted to zero in immunized monkeys. During the first two years following the mass TTI of the colony, the total mortality rate dropped by 42.2% [Kessler et al., 1988]. Despite initial predictions that tetanus toxoid boosters might be necessary every five years [Kessler et al., 1988], it has not been necessary to give additional boosters of tetanus toxoid to maintain protection against infection. Tests run in 2004 on the original cohort of monkeys immunized in 1985 and 1986 showed that 93.3% (14/15) of the surviving animals had protective tetanus antibody titers at the ages of 20–23 years, well

beyond the life expectancy of the Cayo Santiago rhesus macaques [Kessler et al., 2006]. The authors concluded that two doses of Supertet[®] given one year apart were sufficient to provide life-long immunity to tetanus in rhesus monkeys based on observations, antitoxin titers and because the CDC states that immunization with tetanus toxoid yielding protective levels of antitoxin titers has an efficacy of virtually 100% [Kessler et al., 2006; Wassilak & Kretsinger, 2009; Available online at: www.cdc.gov/vaccines/pubs/pink-book/tetanus.html].

Although the initial effects of the TTI program on total and tetanus mortality have been documented, the long-term demographic effects of preventing tetanus infections in the Cayo Santiago macaques have only been hypothesized [Kessler et al., 1988]. These hypotheses were that: (1) the long-term fitness of the population (λ) would increase due to an increase in survival of adult individuals, (2) differences in survival among social groups would decrease due to a reduction in group-specific tetanus-related mortality, and (3) the age distribution of the population would shift toward older individuals as a consequence of an increase in survivorship and life expectancy [Kessler et al., 1988]. It was also hypothesized that female reproductive value would increase as a consequence of the increase in survivorship. In this study, the longitudinal demographic data base of the Cayo Santiago rhesus macaque population was used to parameterize matrix projection models and to run a life table response experiment (LTRE) in order to analyze the long-term demographic effects of the TTI program of 1985 on this rhesus macaque population and to test the stated hypotheses.

METHODS

All research procedures utilizing the Cayo Santiago monkeys are approved by the Caribbean Primate Research Center (CPRC) and the Institutional Animal Care and Use Committee of the University of Puerto Rico, Medical Sciences Campus, in accordance with USDA regulations and NIH guidelines. This research adhered to the American Society of Primatologists principles for the ethical treatment of primates.

Study Population

Cayo Santiago, a 15.2-ha forested island consisting of the Big and Small Cay (key) connected by an isthmus, is located 1 km off the southeastern coast of Puerto Rico (18°09'N, 65°44'W). The average daily temperature is 28°C, relative humidity ranges between 60% and 75%, and annual rainfall averages 163 cm [Rawlins & Kessler, 1985]. The island has been occupied by a provisioned population of free-ranging rhesus monkeys (*Macaca mulatta*) since 1938 [Rawlins & Kessler, 1986a]. The colony has been used for a variety of behavioral and biomedical studies over the past 75 years [Gonzalez, 2013]. Since 1956, the monkeys have been provisioned with commercial monkey chow at a rate of approximately 0.23/kg/animal/day [Sade et al., 1985]. Filtered chlorinated rain water is available ad libitum. Demographic data are based on detailed life histories kept on over 10,000 individual current and former members of the colony through a daily census conducted by a team of experienced observers (census takers), researchers, research assistants and graduate students. Animal information includes identity, date of birth, sex, maternity, maternal lineage (family), social group(s), parity (if applicable), dominance rank, morbidity, mortality, age at death, and cause of death (if known). Genotype and paternity are known on over 2,300

monkeys born into the colony since 1992 [Albers & Widdig, 2013]. The number of naturally-formed social groups in the population varied from four to eight. Births, deaths, changes in group membership, and missing monkeys were generally noted within two days of occurrence. Moribund and severely injured animals were either permanently removed from the island by the veterinary staff for treatment or euthanized in accordance with guidelines of the American Veterinary Medical Association. Veterinary intervention was minimal throughout the study period except during the annual roundups when monkeys were given physical examinations and had blood samples taken. Those monkeys transferred to the CPRC's Sabana Seca Field Station were tested for tuberculosis, given anthelmintics and screened for viruses upon arrival, thus serving as sentinels for the Cayo Santiago population.

Tetanus Toxoid Inoculation Program

Research with human and veterinary tetanus toxoid products at the CPRC in rhesus monkeys [Hardegree et al., 1982] and experiences dealing with tetanus in various monkey species at its Sabana Seca Field Station [Kessler & Brown, 1979; Kessler & Martinez, 1979; Kessler et al., 1982; Kessler & Rawlins, 1983] indicated that an adsorbed veterinary tetanus toxoid product (Supertet[®] with Havlogen[®], Haver-Lockhart Laboratories [now Intervet, Inc., Millsboro, DE]) would induce the highest levels of circulating tetanus antitoxin and provide protection from infection in most rhesus monkeys following a single 0.5 ml intramuscular dose. However, based on these studies in order to induce long-term protection against tetanus in all monkeys, at least two doses of tetanus toxoid would be required [Hardegree et al., 1982].

Because the management policy for Cayo Santiago allows the colony to be captured only once per year (annual roundup), in order to conduct an effective mass TTI program, primary inoculations had to be given one year and secondary (boosters) the following year. Thus, between January and March 1985, all monkeys in the colony ($n = 706$), except for six not captured and infants of the 1985 birth crop, were given a 0.5 ml deep intramuscular dose of Supertet[®] with Havlogen[®] (lot 51052) in a thigh. This was the first time that any Cayo Santiago monkeys had received TTI except for the 1973 experiment in which only 23 animals were inoculated [Kessler et al., 1988]. During the 1986 roundup, the yearlings born in 1985 and five of the six monkeys not caught in 1985 were given their primary inoculations using the same toxoid product (lot 51056-E). Remaining animals received boosters using the same dose as the primary inoculation. Again, newborn infants were not inoculated. During the 1987 roundup, yearling and two-year-old monkeys received primary and booster inoculations (lot 51059-D), respectively. This protocol using Supertet[®] with Havlogen[®] has been followed ever since in subsequent cohorts of monkeys i.e. during the annual roundups of the colony all yearlings have received a primary dose of tetanus toxoid followed by a booster at two years of age [Kessler et al., 1988]. Subsequent observations revealed that this inoculation protocol was completely protective and appeared to provide life-long immunity against tetanus infections [Kessler et al., 2006].

Demographic Data Collection and Parameterization

For this study, demographic data on all monkeys alive from 1973 to 1996 were used (2,204 males; 2,074 females). These data were divided into 12 pre-TTI years (1973–1984) and 12 post-TTI years (1985–1996). Data prior to 1973 were not used in order to avoid inconsistencies between information on dead individuals and culled ones [Sade et al., 1985]. There were no changes in the management of the colony from 1973 through 1983 and the colony was allowed to increase without any intervention or culling. From 1984 to 1995 there were periodic culls of intact social groups. Removing multiple social groups during the same annual roundup, as occurred in 1984, extended the usual length of intervention. This affected the behavior of mother-infant pairs by making some mothers more protective [Berman, 1989] but did not significantly affect colony reproduction or mortality rates allowing a comparison of demographics over equal periods pre- and post-TTI [Hernández-Pacheco et al., 2013]. From 1973 to 1996, the mean number of monkeys on the island was 967 ± 366 (mean $\pm$ SD). During the pre-TTI period, the number of females ranged from 169 to 653 while during the post-TTI period females ranged from 381 to 771. During the pre-TTI period, the number of males ranged from 176 to 663 and during the post-TTI period from 427 to 829.

Per Hernández-Pacheco and colleagues [2013], a record was compiled of (i) the total number of monkeys alive in the population each year, (ii) the age of sexual maturity of all females (age at first delivery), (iii) the total number of females that gave birth to females at specific ages, (iv) the age at death or age when removed (transferred from Cayo Santiago to the CPRC's Sabana Seca Field Station [culled]), and (v) the natal social group of each individual. Age-specific mortality (q_x)—the probability of dying at a specific age x —was defined as the number of individuals that exited the colony by death (d_x) divided by the number of live individuals in the same age class at the start of the time period. Age-specific survival rate (P_x)—the probability of surviving from one age class to the next—was defined as $1 - q_x$. The probability of surviving from birth to the beginning of age x (l_x) was estimated as the product of P_0 to P_{x-1} . In order to estimate the mean expectation of further life or the age-specific life expectancy (e_x), the proportion of survivors at the mid-point of each time interval or the life table age structure at age x (L_x) was estimated by averaging l_x and l_{x+1} ,

and the cumulative function for each age class (T_x) was estimated as $\sum_{x=n}^k L_x$. Age-specific life expectancy (e_x) was estimated as T_x/l_x . The generation time (G), the time required for the population to increase by a factor of R_0 , was estimated by $\log(R_0)/\log(\lambda)$, where R_0 is the net reproductive rate and λ is the population growth rate. Age-specific fertility of females (F_x) was estimated by dividing the number of newborn females at time t by the number of females of age x alive at time t . Cohort analysis was used to test for temporal variability in vital rates using nonparametric statistics; Mann–Whitney W -test, and Kruskal–Wallis test. Nonparametric statistics were used to avoid any assumptions about the distribution of the mean annual vital rates from the pre-TTI and post-TTI periods. Each cohort was defined as the total number of monkeys born each year. In order to avoid age classes with no representation during the first period of the study, males 12 years of age or older were grouped into one age class, as well as females 14 years of age or older. To test for differences in survival among social groups, the active period of each group was

determined as the period between the first and last cohort recorded in each social group. Only groups with cohorts present during both pre-TTI and post-TTI periods were analyzed (Groups F, I, and L). In order to have the maximum number of groups possible, the analysis was done using data until 1990. Group sizes (mean $\pm$ SD) varied through time between both pre-TTI and post-TTI periods from 91 ± 20 to 125 ± 30 monkeys in group F; 72 ± 37 to 211 ± 30 in group I; and from 45 ± 15 to 116 ± 35 in group L. All data analysis was carried out using the R.3.0.1 packages popbio and pgirmess [Stubben & Milligan 2007, Available online at: <http://www.jstatsoft.org/v22/i11>; Giraudoux, 2013, <http://perso.orange.fr/giraudoux>; R Development Core Team, <http://www.R-project.org>]. Statistical significance was set at $P < .05$.

Demographic Model

To examine differences in long-term population fitness—population growth rate (λ)—, and to carry out a retrospective analysis to quantify the population-level effect of the tetanus toxoid inoculation program, two matrix projection models were parameterized using the data from both pre-TTI and post-TTI periods. Hypothetical annual censuses were compiled from the daily census data of females and a birth-pulse model employing post-breeding censuses was carried out [Caswell, 2001; Hernández-Pacheco et al., 2013]. An age-based Leslie matrix incorporating survival (P_x) and fertility rates (F_x), and also allowing for stasis of the last age class was constructed. Therefore, the transition matrix contains positive data in the first row, the first subdiagonal, and the last entry of the matrix [Caswell, 2001; Dobson & Lyles, 1989]. Due to the lack of significant differences in survival among female individuals 4 years or older, as well as the significant difference in fertility rates between 3 and 4-year-old females and older, the population was divided into five stage classes according to their ages: infants [I] (<1-year-old), yearlings [Y] (1 to <2-year-old), juveniles [J] (2 to <3-year-old), young adults [YA] (3 to <4-year-old), and adults [A] (≥ 4 years) [Hernández-Pacheco et al., 2013]. To account for differences in the proportion of adult individuals belonging to different age categories, estimates of adult mean survival P_A were calculated on the weighted mean based on the number of individuals in each age class. An eigen analysis was carried out and the real dominant eigen value and its corresponding right and left eigenvectors were calculated in order to obtain the asymptotic population growth rate (λ), the stable stage distribution (w), and the reproductive value vector (v), respectively.

In order to statistically test for differences in long-term population fitness, a bootstrap was carried out and 95% confidence intervals were determined for the λ s of each period by randomly selecting individuals from the transition data set, with replacement, until attaining the original sample size. Demographic transitions were calculated from this sample, a transition matrix was constructed, and λ calculated. The entire procedure was repeated 1,000 times and asymmetric 95% confidence limits were determined from the 2.5th and 97.5th percentiles of the data. In addition, a two-sample Kolmogorov–Smirnov test was carried out to test the null hypothesis that the two generated λ distributions were drawn from the same distribution. To test whether survival or fertility rates were density-dependent, and thus changed as a function of population size, regression analyses between both vital rates and the number of females were carried [Hernández-Pacheco et al., 2013]. Male data were not included in the matrix projection model, as our model assumes that female life history

schedules determine population dynamics as female reproductive success is not limited by the number of adult males [Caughley, 1977; Lawler, 2011; Rawlins & Kessler, 1986b]. Although information on natal social group for each male is available, male rhesus periodically emigrate among groups. Thus, males were not included to test for differences in survival among social groups.

A LTRE was performed [Caswell, 1996, 2001] as a retrospective analysis to quantify the population-level effect of the tetanus toxoid inoculation program in relation to the years prior to the initiation of the program. For this, two matrices were constructed; one with the data belonging to years prior to 1985 - the reference matrix $A(r)$ - and one with the data belonging to post-TTI years - the treatment matrix $A(t)$ [for a detailed description on LTRE, see Caswell, 1996, 2001].

RESULTS

Mass inoculation of the Cayo Santiago colony with tetanus toxoid had significant effects on the demography of the population as a direct consequence of a reduction in mortality. For the period of this study (1973–1996), the mean annual total mortality rate decreased from 7.22% prior to inoculation to 4.78% following inoculation or a decrease of 33.8% (Mann–Whitney W-test = 23.5, $**P < 0.01$, $N = 24$) (Table I). During the pre-TTI period the female population increased from 173 to 652 and exhibited a mean annual net population growth rate (λ) of 1.128 (range: 1.062–1.165). The variation in λ indicated the population experienced annual net growth rates of up to 16.5%. During this pre-TTI period, age-specific mean survival rates for females were 88% in infants, 96% in yearlings, 98% in juveniles, and 94% and 95% in young adults and adults, respectively (Table II). During the post-TTI period, during which a total of 661 females were culled from the population as part of the colony management plan, the female population increased from 379 to 499 exhibiting an increase in mean λ of 1.134 (range: 1.094–1.179). During this period age-specific mean survival for females was 88% in infants, 96% in yearlings, 97% in juveniles, and 98% and 97% in young adults and adults, respectively (Table II). Although the relative increase in mean survival among young adult females was higher than that of adult females, the LTRE confirmed that survival of adult females was the life cycle event that contributed most to the observed increase in λ post-TTI (Table III). Positive values in Table III indicate life cycle transitions that contributed to the increase in population growth rate and negative values indicate transitions that slowed down population increase after inoculation. In fact, 59% of the effect on λ came from changes in survival of young adult and adult females, and, of those changes, 78% came exclusively from changes in the survival of adult females (Table III). Based on adult cohort analysis (ages 4–14), the adult female population exhibited a significant increase in age-specific mean annual survival following 1985 (Mann–Whitney W-test = 10039, $P < 0.05$, $N = 264$) (Fig. 1). No density-dependence in survival was found. In contrast, a negative linear relationship between fertility rates of young adults and adults and the total number of adult females was found (young adults; $y = -0.0004x + 0.218$, $R^2 = 0.13$; adults; $y = -0.0002x + 0.384$, $R^2 = 0.15$, respectively).

The frequency distribution of λ generated by bootstrapping the transition data from both periods showed more variability during the pre-TTI period, ranging from 1.099 to 1.137 and

95% CI = 1.109, 1.131, while post-TTI λ distribution ranged from 1.111 to 1.134 and 95% CI = 1.115, 1.130 (Fig. 2). These changes were significant (Kolmogorov–Smirnov K-S test: $D = 0.308$; $P < 0.0001$, $N = 2000$) causing a positive shift toward higher values of λ during the post-TTI period (Fig. 2).

No significant difference in mean age-specific survival among social groups was found during the post-TTI period (Kruskal–Wallis chi-squared = 2.101, $df = 2$, $P = 0.350$, $N = 45$). Mean age-specific survival in social groups I and L increased significantly following inoculation (Mann–Whitney W-test = 183, $P < 0.005$, $N = 30$; Mann–Whitney W-test = 190, $P < 0.005$, $N = 30$, respectively, Supplementary Appendix A). During the pre-TTI period, groups F, I, and L exhibited age-specific survival ranging from 0.838 to 0.984, 0.512 to 1.000, and 0.819 to 1.000, respectively. During the post-TTI period groups F, I, and L exhibited age-specific survival ranging from 0.898 to 1.000, 0.897 to 1.000, and 0.917 to 1.000, respectively (Supplementary Appendix A). The age-specific mean survival distributions of each group had medians of 0.949, 0.941, and 0.882 during pre-TTI and medians of 0.973, 0.977, and 0.986 during post-TTI, respectively.

The age distribution of the Cayo Santiago population shifted toward older individuals following TTI (Fig. 3). During the post-TTI period, the proportion of male infants decreased from 17.5% to 16.9%, as well as the proportion of yearlings, juveniles and young adults that decreased from 14.4% to 14.0%, 12.3% to 11.4%, and 10.6% to 9.50%, respectively. In contrast, the proportion of 4 year old and older males increased from 45.1% to 48.2% (Fig. 3). Similarly, during the post-TTI period the proportion of female infants decreased from 17.3% to 16.5%, as well as the proportion of yearlings, juveniles and young adults that decreased from 14.2% to 13.6%, 12.0% to 11.3%, and 10.1% to 9.60%, respectively (Fig. 3) while the proportion of 4-year-old and older females increased from 46.4% to 49.0% (Fig. 3).

The age-specific survivorship (l_x) also increased throughout adulthood (Fig. 4A, C; Supplementary Appendix B, C). This increase resulted in a higher life expectancy and in a longer generation time during the post-TTI period. The life expectancy at birth increased from 11.2 to 14.4 years (28.6%) for males and from 12.9 to 16.3 years (26.4%) for females. For monkeys reaching yearling status, when life expectancy peaked, it increased even more, from 8.4 to 11.5 years (36.9%) for males and from 13.7 to 17.5 years (27.7%) for females (Fig. 4B, D; Supplementary Appendix B, C). Tetanus deaths peaked at 4–6 years of age in males and at 2–4 years of age in females [Rawlins & Kessler, 1982]. Therefore, differences in survival among those specific age classes were tested. Mean annual survival of 4–6 year old males increased significantly following TTI (Mann–Whitney W-test = 873, $P < 0.05$, $N = 72$), as did survival of 2–4 year old females (Mann–Whitney W-test = 835, $P < 0.05$, $N = 72$). During the pre-TTI period, the oldest male attained 27 years while the oldest female reached 28 years of age (Fig. 3; Supplementary Appendix B1, C1) while during the post-TTI period, the oldest male reached 29 years and the oldest female reached 31 years of age (Fig. 3; Supplementary Appendix B2, C2).

The net reproductive rate (R_0) also increased from 5.03 to 8.50 causing the generation time to increase from 13.5 years to 17.0 years. The reproductive value (RV) of females increased

among ages of maximum value (Fig. 5). The RV curve shows that RV peaked at 4–6 years of age during both periods. However, age-specific RV was higher during the post-TTI period.

DISCUSSION

The mass tetanus toxoid inoculation of the Cayo Santiago rhesus macaque colony started in 1985, allowed, for the first time, a chance to study the long-term demographic impact of a naturally-occurring infectious disease on an entire rhesus population [Hernández-Pacheco et al., 2013; Kessler et al., 2006; Southwick, 1989] and provided an opportunity to test hypotheses put forth 25 years ago. Our analysis supports the three hypothesis by Kessler and colleagues [1988] that following inoculation in 1985 (i) the long-term fitness of the population (λ) would increase, (ii) the difference in survival rates previously reported among social groups would decrease, and (iii) the population's age distribution would shift toward older individuals by increasing survival of adults and life expectancy [Kessler et al., 1988].

Total deaths and mortality rates in the colony decreased significantly after tetanus toxoid inoculation and only two tetanus cases (one fatal and one nonfatal) were observed in two of six monkeys that missed TTI in 1985. No additional cases were seen among the 685 deaths occurring in the colony from 1985 through 1996. This compares with 57 fatal cases among 67 total tetanus cases observed from 1976 through 1981 prior to TTI [Kessler et al., 1988; Rawlins & Kessler, 1982]. Thus, the TTI program begun in 1985, for all intents and purposes, eradicated tetanus infections in the colony as similar programs have done in other outdoor colonies of monkeys [Goodwin et al., 1987; Goss, 1942; Kessler & Brown, 1979; Kessler & Martinez, 1979; Springer et al., 2009.] The reduction in the total mortality rate was greater than expected from the number of observed tetanus cases in two previous studies of the disease in the colony [Kessler et al., 1988; Rawlins & Kessler, 1982] but less than the 42.2% decrease in the total mortality rate in the two years immediately following TTI [Kessler et al., 1988]. This could be due to other causes of mortality over the lifetime of these monkeys. Unfortunately, determining the cause of death is often difficult because monkey carcasses rapidly decompose in the tropical heat [DiGiacomo & Missakian, 1972].

The long-term population fitness (λ) is a function of both survival and fertility rates. Although survival increased significantly following TTI, fertility did not due to negative density-dependence [Hernández-Pacheco et al., 2013]. This expected negative effect on λ was confirmed by the LTRE where fertilities had a negative value in the contribution matrix given the observed difference in mean population density between the two periods. Although a negative linear relationship between fertility rates of young adults and adults, and the total number of adult females was found, a significant increase in λ was still observed following TTI. As hypothesized [Kessler et al., 1988] and confirmed by the LTRE, this increase in λ resulted primarily from the observed increase in adult female survival. This is because survival of adult females was the life cycle event showing a significant increase, the highest sensitivity value, and because it was unaffected by changes in population density. In fact, no difference in temporal variation of total mean survival rate as a function of population density, or any potentially catastrophic external events, such as hurricanes, was found previously for the same time period [Hernández-Pacheco et al., 2013]. Thus, the

changes in survival rate are considered a direct consequence of the eradication of tetanus infections rather than the result of any changes in management, population density or environmental variability. Increased survival was expected as older monkeys on Cayo Santiago had a high tetanus mortality rate, and the cumulative risk of tetanus deaths in aging animals was 53% by 13 years of age [Kessler et al., 1988].

Significant differences in survival rates among social groups were not observed during the post-TTI period. Previous studies found that tetanus deaths on Cayo Santiago accounted for up to a third of all mortality in Group L and the tetanus mortality rate in Group L was 9.6 times higher than that of the group with the lowest rate [Rawlins & Kessler, 1982; Rawlins & Kessler, 1986b]. The significantly different rate of growth among the six social groups was due to the lower survival rate in Group L. Higher tetanus mortality and lower survival rates for Group L were not related to greater levels of aggression. Kaplan [1977] showed the frequency of both aggression and affiliative encounters within Group L was significantly below that observed in the other social groups, so it is unlikely that Group L sustained relatively greater wounding as the primary cause of tetanus infections [Rawlins & Kessler, 1982; Springer et al., 2009]. The comparatively high incidence of tetanus in Group L was probably due to its restricted range on the Small Cay [Lauer, 1979] where, from 1970 to 1972, a horse was corralled [Sade et al., 1985]. Because horses excrete high concentrations of the vegetative form of *Clostridium tetani* in their manure [Gillespie & Timoney, 1988], the high tetanus mortality rate in Group L compared to other groups could have been a direct result of its ranging and foraging where the horse was confined [Rawlins & Kessler, 1982]. With tetanus playing such an important role in survival (mortality) of individuals in Group L, TTI decreased the differences in mortality rates among the social groups to non-significant levels. Reduction of differential mortality in turn impacts the relative genetic contribution of the various social groups and their constituent matriline [McMillan, 1986; McMillan & Duggleby, 1981].

The age distribution of the Cayo Santiago population shifted so that the relative number of monkeys attaining older age increased. This resulted from the increase in survival among adults and the consequent increase in life expectancy. The age-specific tetanus mortality rate approached or exceeded the total mortality rate of 6.77% for the Cayo Santiago population after eight years of age, and tetanus accounted for 43% of all deaths in monkeys 16 years of age [Kessler et al., 1988]. Besides tetanus, other potential causes of death among geriatric individuals might have included infections from fight wounds; post-partum infections; arthritis leading to debility, muscle wastage and inability to remain with their social group; chase injuries suffered while traversing the forested and rocky environment; enteritis; and dehydration. However, it is unlikely that the frequency of such events changed through time as management practices, including veterinary care, were kept constant.

Without the risk of tetanus, monkeys had a greater chance of attaining older ages. This change was reflected in the age distribution of the population and in the age-specific survivorship curves (l_x) for both males and females when compared to the pre-TTI period. Moreover, tetanus deaths peaked at young age in females, so that the prevention of tetanus in females significantly enhanced the probability of individuals reaching maturity and shifting the age distribution toward older ages.

Even with a difference in average female density among the different periods, the R_0 and the age-specific RV of females increased following TTI. This is different from the observed decrease in mean age-specific fertility (F_x) during the post-TTI period. Net reproductive rate and age-specific RV are given by F_x , as well as by survivorship (l_x) [Chapais & Schulman, 1980]. These findings suggest that the increase in survivorship following TTI also had significant effects on the reproductive parameters of females highlighting the significant consequences of the TTI program on the viability of the population.

Implications for Colony Management

The eradication of tetanus infections in the Cayo Santiago population has had significant long-term consequences on the demography and life expectancy of these monkeys. At the population level, the viability of the colony has been improved. Its genetic diversity has likely been enhanced by the elimination of differential mortality among its social groups and by increased genetic representation of the more highly-affected matriline. From a management perspective, the generation of a revised life table for the Cayo Santiago macaques post-TTI, makes it possible to effectively model the colony for future management and population control. Importantly, the elimination of clinical tetanus infections through mass TTI has greatly improved the quality of life for the animals during the past 30 years by preventing a common cause of severe morbidity and mortality. This study supports the general consensus that prophylactic tetanus toxoid inoculations should be given to all nonhuman primates residing in outdoor facilities, especially those in contact with soil [DiGiacomo & Missakian, 1972; Goodwin et al., 1987; Kessler & Brown, 1979; Kessler et al., 2006; Nakano et al., 2012; Springer et al., 2009].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Mean annual survival probability of adult female cohorts from Cayo Santiago during the pre-tetanus toxoid inoculation period (white) and post-tetanus toxoid inoculation period (grey). Mann–Whitney W-test = 10039, $P < 0.05$, $N = 264$.

Fig. 2.

Frequency distribution of population growth rates of female rhesus macaques for pre-tetanus toxoid inoculation period (white) and post-tetanus toxoid inoculation period (bars with lines) (K-S test: $D = 0.308$; $P < 0.0001$, $N = 2000$).

Fig. 3. Life table age distribution of male and female rhesus macaques during the pre-tetanus toxoid inoculation (black) and post-tetanus toxoid inoculation (white) periods.

Fig. 4. Standardized survivorship curve (l_x) (A and C) and life expectancy (e_x) (B and D) of female and male rhesus monkeys during the pre-tetanus toxoid inoculation and post-tetanus toxoid inoculation periods. Black lines represents pre-tetanus toxoid inoculation periods, dashed lines represent post-TTI periods.

Fig. 5. Reproductive value (RV) curve for pre-tetanus toxoid inoculation (solid line) and post-tetanus toxoid inoculation (dashed line) periods.

TABLE I

Mean Annual Mortality Rate of all Monkeys During pre-(1973–1984) and post-(1985–1996) Tetanus Toxoid Inoculation (TTI) Periods

Pre-TTI	Mortality rate	Post-TTI	Mortality rate
1973	0.110	1985	0.041
1974	0.085	1986	0.034
1975	0.098	1987	0.049
1976	0.081	1988	0.040
1977	0.066	1989	0.045
1978	0.066	1990	0.041
1979	0.041	1991	0.043
1980	0.069	1992	0.060
1981	0.072	1993	0.064
1982	0.067	1994	0.038
1983	0.072	1995	0.047
1984	0.038	1996	0.072
Mean	0.072	Mean	0.048
SD	0.02	SD	0.012
Median	0.070	Median	0.044
95%CI	0.039, 0.107	95%CI	0.035, 0.070

Note: Proportions reported. SD and CI stand for standard deviation and confidence intervals. Mann-Whitney W-test = 23.5, $P < 0.01$, $N = 24$.

TABLE II

Demographic Matrices Showing Mean Transition Probabilities, Stage Specific Mortality (Q_x), and Number of Infants (I), Yearlings (Y), Juveniles (J), Young Adults (YA), and Adult (A) Female Rhesus (N_x) for pre-TTI and post-TTI Periods

	Age class at time t				
	I	Y	J	YA	A
Age class at time t + 1					
1973–1984					
I	0.000	0.000	0.000	0.190	0.344
Y	0.875	0.000	0.000	0.000	0.000
J	0.000	0.957	0.000	0.000	0.000
YA	0.000	0.000	0.983	0.000	0.000
A	0.000	0.000	0.000	0.939	0.945
Q_x	0.125	0.043	0.017	0.061	0.055
N_x	762	604	518	427	2016
1985–1996					
I	0.000	0.000	0.000	0.067	0.331
Y	0.877	0.000	0.000	0.000	0.000
J	0.000	0.956	0.000	0.000	0.000
YA	0.000	0.000	0.973	0.000	0.000
A	0.000	0.000	0.000	0.976	0.969
Q_x	0.093	0.080	0.038	0.031	0.031
N_x	1,139	962	771	667	3,388

TABLE III

Matrix of Contribution from a Life Table Response Experiment (LTRE) Showing Contributions of Each Life Cycle Transition of Rhesus Macaques to Population Increase Following the Tetanus Toxoid Inoculation in 1985.

	Age class at time t				
	I	Y	J	YA	A
Age class at time t + 1					
I	0.0000	0.0000	0.0000	-0.0069	-0.0042
Y	0.0002	0.0000	0.0000	0.0000	0.0000
J	0.0000	-0.0001	0.0000	0.0000	0.0000
YA	0.0000	0.0000	-0.0011	0.0000	0.0000
A	0.0000	0.0000	0.0000	0.0040	0.0140

Note: Positive values indicate life cycle transitions that contributed to the increase in population growth rate and negative values indicate transitions that slowed down population increase after inoculation. Highlighted Life Cycle Transition Contributed the Most to Changes in Population Growth Rate Following Inoculation. Age Classes are Infants (I), Yearlings (Y), Juveniles (J), Young Adults (YA), and Adult (A) Female Rhesus