EFFECT OF RED IMPORTED FIRE ANTS ON IMMUNOCOMPETENCE OF NORTHERN BOBWHITE CHICKS

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Abstract

Northern bobwhites can suffer high mortality due to stings from red imported fire ants while hatching. We conducted a study on wild northern bobwhites to determine if chicks that survived natural exposure to red imported fire ants at hatching suffered reduced growth or development of immunocompetence. We compared 22-day chick masses and measures of humoral and cell-mediated immunity between chicks exposed to natural levels of red imported fire ants at hatching and chicks from nests where ants were exterminated. No variables differed between treatments. These results suggest that growth and immunocompetence of northern bobwhite chicks are probably not affected greatly by red imported fire ant stings at hatching. Sublethal effects such as reduced growth can be demonstrated under laboratory conditions, but may occur only rarely under natural conditions due to the rapid recruitment of red imported fire ants to food sources and subsequent rapid accumulation of stings by chicks.

Introduction

The red imported fire ant (*Solenopsis invicta*) is a pest species accidentally introduced into the United States from South America between 1933 and 1941 (Buren 1972, Rhoades 1977). Its range in the United States rapidly expanded and now encompasses more than 1 million km^2 (Williams 1994). The primary diet of these ants is insects and other small invertebrates (reviewed in Vinson and Greenberg 1986); however, they frequently attack and kill vertebrates (reviewed in Allen et al. 1994), including nestling wood ducks (*Aix sponsa*) (Ridlehuber 1982), cliff swallows (*Hirundo pyrrhonota*) (Sikes and Arnold 1986), least terns (*Sterna antillarum*) (Lockley 1995), and northern bobwhites (Mueller et al. 1999).

Before the invasion and spread of red imported fire ants, researchers found that native fire ants (genus *Solenopsis*), such as *S. geminata* and *S. richteri*, killed 4-9% of hatching northern bobwhite (Stoddard 1931, Travis 1938, Johnson 1961). However, the impact of red imported fire ants to northern bobwhite is more severe than that of native fire ants. Mueller et al. (1999) estimated that 38.1% of all northern bobwhites that hatched in 1997-98 died as a result of red imported fire ant stings inflicted at the time of hatching. This estimate of the proportion of northern bobwhites killed may have been negatively biased if an assumption, that all mortalities

caused by ant stings at hatching occurred within 21 days, was violated. This assumption was required because chick survival was measured 21 days after each brood hatched.

Could red imported fire ant stings to chicks at hatching cause mortality >21 days after hatching? Giuliano et al. (1996) found northern bobwhite chicks that survived the most severe exposures to red imported fire ants in a laboratory study weighed 12% less than controls after 9 days (the duration of the experiment). However, 77% of the chicks at this level of exposure died (Giuliano et al. 1996). Thus, under laboratory conditions, most chicks died when exposed to a level of red imported fire ant stings that reduced growth. Wild northern bobwhites, however, face greater difficulty in acquiring optimal nutrition, and the effect of red imported fire ant stings may be more severe. For example, chicks in lab studies might be capable of obtaining optimal nutrition after being partially blinded by red imported fire ant stings because feed is easily found and provided ad libitum. Thus, levels of red imported fire ant exposure equivalent to the study by Giuliano et al. (1996) would probably cause greater mortality and reductions in growth in wild northern bobwhites; in addition, reductions in growth might occur over a broader range of red imported fire ant exposure.

Giuliano et al. (1996) did not describe the mechanism by which red imported fire ant stings reduced growth of northern bobwhite chicks. However, Pedersen et al. (1996) reported that red imported fire ants often stung the eyelids, legs, and toes of northern bobwhite chicks. Stings to the eyelid usually caused the eye to swell closed, and stings to the leg or foot sometimes caused swelling that prevented normal movement. If stings result in reduced intake of protein, chick growth, development of the bursa of Fabricius and spleen, and cell-mediated immunity might be suppressed (Lochmiller et al. 1993). Thus, chicks that survive stings from red imported fire ants while hatching may be unable to acquire an adequate ration of high-protein foods, leading to slower growth, and resulting in greater susceptibility to pathogens for weeks afterward.

To test whether red imported fire ant stings at hatching could potentially influence wild northern bobwhite survival >21 days after hatching, we compared 22-day body mass, cell-mediated immunity, and humoral immunity between northern bobwhite chicks that hatched from nests with natural levels of red imported fire ants and those from nests where ants were exterminated. These chicks left the experimentally treated areas around each nest soon after hatching and thereafter occupied the same natural environment with unmanipulated red imported

fire ant populations.

Methods

Field Techniques

Mueller et al. (1999) provided a detailed description of the experimental treatments. Briefly, we found nests by monitoring radiomarked northern bobwhite hens from late April until mid July 1998. When a nest was found, it was randomly assigned to either receive a fire ant suppression treatment or be a control. For the fire ant suppression treatment, a fire ant insecticide was applied (1.7 kilograms/ha of Amdro®, American Cyanamid Company, Wayne, New Jersey) using a handheld broadcast spreader. Treatments were applied to a 60- x 60-m area centered on the nest 3 weeks before the expected hatch date followed by a retreatment 2 weeks later to the 20- x 20-m core area around the nest. Five variables were compared between chicks from treated and control nests: hatching success, 21-day survival, 22-day body mass, humoral immunity, and cell-mediated immunity. Methods and results for comparisons of hatching success and 21-day survival were reported in Mueller et al. (1999). The following describes my methods for capturing chicks and measuring 22-day body mass, humoral immunity, and cell-mediated immunity.

Chick Capture

Broods were captured 22 days after hatching by netting at night. Coveys were found by tracking the signal from the hen's radio transmitter and illuminating the brood with a handheld 500,000 candlepower spotlight. Broods were captured using a handheld net (Mighty Net, Animal Management, Inc., Heafford Junction, Wisconsin; netting was replaced with 1/4 inch mesh). Chicks were weighed (± 0.1 g), banded with aluminum legbands, and radiomarked with transmitters weighing 0.7 g (American Wildlife Enterprises, Montacello, Florida). To assess chick immunocompetence, chicks were recaptured after 5 and 6 days by locating them using telemetry and capturing them by hand or net.

Humoral Immunity

Humoral immunity was assessed by measuring antibody production in response to an injection of sheep red blood cells. Chicks were inoculated intramuscularly with 0.5 mL of 5% sheep red blood cells (Colorado Serum Company, Denver, Colorado) in phosphate buffered saline (Lochmiller et al. 1993) when initially captured and radiomarked. Antibody production was assessed after 6 days by pricking the ulnar vein and collecting blood into capillary tubes. Blood was kept shaded at ambient temperature for up to 4 hr and then centrifuged for 10 min. Serum was pipetted from the capillary tubes, placed into cryovials, stored at -20°C for ≤9 weeks, shipped on dry ice, and then stored at -84°C for 6 months until assayed.

Antibody responses to the sheep red blood cell inoculation was measured using a microhemagglutination assay (Wegmann and Smithies 1966). As described by Lochmiller et al. (1993), serial 2-fold dilutions of heat-inactivated serum (56°C for 30 min) in phosphate-buffered saline were mixed with an equal volume of 1% sheep red blood cell solution in phosphate-buffered saline and incubated at 40°C for 1 hr. Titers were expressed as the $\log_2$ of the reciprocal of the highest dilution of serum showing a positive hemagglutination.

Cell-mediated Immunity

Cell-mediated immunity was assessed by measuring wing web inflammation caused by an intradermal injection of the T-cell mitogen phytohemagglutinin (PHA-P; Sigma, St. Louis, Missouri). The thickness of each wing web (± 0.01 mm) of 27-day-old chicks was measured using a digital thickness gauge (Digimatic Thickness Gage, model 547-520, Mitutoyo Corporation, Takatsu-ku, Kawasaki, Kanagawa, Japan). The right wing web was injected with 0.5 mg of PHA-P in 0.1 mL of phosphate buffered saline to stimulate inflammation and the left wing web with 0.1 mL of phosphate buffered saline to serve as a control (Lochmiller et al. 1993). After 24 hr, the chicks were recaptured and wing-web thickness was remeasured. Wing-web swelling was calculated as the difference in wing-web thickness before and 1 day after injection. The cell-mediated immune response, termed the wing-web index, was the difference in swelling between the right and left wing webs (Lochmiller et al. 1993).

Statistical Analysis

Brood means for 22-day chick masses, antibody titers, and wing-web indexes were compared between treatments using t-tests. Brood means rather than means of all chicks were used because experimental treatments were applied to nests, not eggs or chicks. Thus, variables for chicks within a brood were probably correlated. The assumptions of normality and homoscedasticity were assessed where appropriate using the Shapiro and Wilk (1965) and Levene (1960) tests, respectively. Analyses were conducted using SPSS for Windows, release 6.0 (Norušis 1993) and GPOWER, version 2.0f (Erdfelder et al. 1996). Statistical significance was determined with á = 0.05.

Results

WEcaptured and radiomarked 37 chicks from 11 nests. Of these, 9 lost their radio transmitters, 6 died, 5 could not be found and were presumed to either have a faulty radio transmitter or have been depredated, and 17 were successfully monitored until they were 28 days old.

No differences were detected between chicks that hatched from treated and control nests for 22-day mass, humoral immunity, or cell-mediated immunity (Table 3.1). Chick masses ranged from 18.7 to 54.8 g. The heaviest chick failed to express a primary antibody response to sheep red blood cell immunization and had the third lowest wing-web index. Excluding this chick, hemagglutinating antibody titers ($\log_2$) ranged from 2 to 16 and wing-web indexes ranged from 0.21 to 1.24 mm. Mass was not linearly related to individual chick antibody titers ($F_{1, 9} = 0.13$, $P = 0.726$) or wing-web indexes ($F_{1, 14} = 0.08$, $P = 0.788$).

Table 3.1. Masses at 22 days, sheep red blood cell antibody titers, and wing-web indexes for northern bobwhite chicks (*N*) from nests (*n*) treated with a fire ant insecticide and controls, Refugio County, Texas, 1998.

Variable	Treated				Control						
	N	*n*	_	SE	*N*	*n*	_	SE	*t*	*P*	Power
Mass (g)	22	6	38.1	2.6	15	5	35.4	3.1	0.69	0.51	0.09
Antibody titer (log_2)	6	4	8.5	2.9	5	3	13.7	2.3	1.32	0.25	0.18
Wing-web index (mm)	10	5	0.74	0.15	6	3	0.91	0.13	0.77	0.47	0.10

Discussion

Body mass at 22 days and immunocompetence of northern bobwhite chicks were not affected by exposure to natural levels of red imported fire ants at hatching. Few comparable data were available to determine the reasonableness of the observed values, so WEcalculated an optimal 22-day body mass using an equation derived by Blem and Zara (1980) for northern bobwhites fed ad libitum on a 30% protein diet. Blem and Zara (1980) stated that their asymptotic weight of 205 g was undoubtedly greater than reported previously for northern bobwhites due to the genetic strain used. For a more realistic asymptotic weight, WEused the average mass of 175 northern bobwhite captured on my study area in February 1997 and 1998 (_ = 169 g). This mean should be a reasonable estimate of asymptotic weight because body mass of northern bobwhites peaks in February when averaged by gender (Roseberry and Klimstra 1971). Using the other growth parameters of Blem and Zara (1980) and the site-specific estimate of asymptotic size, the predicted 22-day body mass of northern bobwhite was 37.9 g. Chicks from treated nests and control nests attained 100.5% and 93.4% of this predicted 22-day body mass, respectively.

The probability of falsely finding no difference between treatments in body mass and immunocompetence was high due to the small sample size. For example, the power for detecting the observed difference in body masses between treatments was 0.09 (Table 3.1). The effect size for this test was 0.41, which is generally considered a small- to medium-sized effect (Cohen

1977). For a more biologically relevant assessment of power, WEcalculated the power of detecting a difference between body mass treatment means of 10 g. Nestler et al. (1942) reported that 21-day body mass of northern bobwhites fed either a 22% or 30% protein diet differed by about 10 g. The power of detecting a 10-g difference with the sample sizes (n = 5 and 6), standard deviation (6.7), and alpha (0.05) of this study was 0.60. Thus, this study had a 60% probability of detecting an effect of the same magnitude as a 22% versus 30% protein diet. These results should be used in context with previous studies to focus future research at the specific mechanisms by which red imported fire ants impact northern bobwhites. While my sample size was inadequate for detecting small differences between treatments, my observed treatment means and previous studies (Giuliano et al. 1996) suggest that growth and immunocompetence of northern bobwhite chicks are probably not affected greatly by red imported fire ant stings at hatching. However, northern bobwhite chick survival is greatly reduced by red imported fire ant stings at hatching (Mueller et al. 1999).

Under laboratory conditions, the effect of red imported fire ant stings on growth of northern bobwhite chicks was small and only detectable under conditions that resulted in 77% mortality (Giuliano et al. 1996). Only a narrow range of red imported fire ant exposure appears to reduce growth without causing death. Thus, WEspeculate that, under natural conditions, few hatching northern bobwhite chicks are exposed to red imported fire ants at a level that is sub-lethal but biologically detrimental.

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