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HEMATOLOGIC RESPONSES IN CAPTIVE WHITE-WINGED DOVES (*ZENAIDA ASIATICA*), INDUCED BY VARIOUS RADIOTRANSMITTER ATTACHMENTS

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ABSTRACT: White blood cell counts, heterophil–lymphocyte ratios, and leukocyte differentials of captive white-winged doves (*Zenaida asiatica*) from Texas equipped with different radiotransmitter attachment packages were monitored. Doves were segregated by gender and age by males, females, and hatching year; individuals housed in 30 large outdoor pens in groups of seven. Treatments consisted of controls, glue-on transmitters, body loop harnesses, surgically implanted intracoelomic transmitters, surgically implanted subcutaneous transmitters, intracoelomic surgery without implants, and subcutaneous surgery without implants. We used multivariate analysis of variance with pen as a blocking variable and gender nested and repeated measures analysis of variance to identify differences among any of the transmitter attachment techniques and the control for dependent variables. We found no difference in blood parameters between transmitter attachment technique versus a control.

Key words: Avian hematology, radiotransmitters, white-winged doves, *Zenaida asiatica.*

INTRODUCTION

The breeding range of white-winged doves (*Zenaida asiatica*) in Texas has historically been restricted to a four-county region (Cameron, Hidalgo, Willacy, and Starr) in the lower Rio Grande Valley (Cottam and Trefethen, 1968). However, white-winged doves have steadily increased the periphery of their range northward, particularly in urban locales, over the last several decades (Cottam and Trefethen, 1968; Small et al., 1989; Hayslette et al., 1996; Waggerman, 1998). Relatively little information regarding natural history dynamics is available for these colonial populations. As a consequence, management strategies specific to local, disjunct populations based on new and aggressive scientific research and methodology are needed (Small et al., 2004a) because of current concern regarding the stability of some recently formed populations of both Mesoamerican migrants and resident individuals (George et al., 1994). The ambulatory nature and fecundity of white-winged doves enables exploitation of anthropogenic alterations, which presents a challenge for contemporary wildlife management. As a consequence, life history, biologic requirements, and management needs have yet to be fully determined.

Telemetry studies provide detailed data, both spatially and temporally, about population demographics (Derleth and Sepik, 1990; Schulz et al., 1996), habitat use (Drobney et al., 1998; Millspaugh et al., 1998), behavior (Howe and Flake, 1988; Rautenstrauch et al., 1998), and other life history characteristics. Researchers using radiotelemetry for wildlife studies often assume that transmitter attachment does not cause aberrant behavior in individuals. If attachment of a transmitter initiates subclinical illness, data collected from an individual may be misleading.

Compared to other vertebrates, birds are unique because they typically show few overt signs of stress or illness in response to disease states (Rosskopf and Woerpel, 1982). As a consequence, diagnostic laboratory tests are preferred in determining, monitoring, and assessing recovery of avian subjects. Rosskopf and Woerffel (1981) discussed the compensatory ability of avian species to "mask" manifestations of disease. Because of this physiologic mechanism, clinical hematologic laboratory evaluation is important in quan-

tifying the severity of the subject's clinical condition (Rosskopf and Woerffel, 1981).

Wildlife research has largely overlooked avian hematology, and few, if any, studies enlist its usefulness as an efficient evaluator of physiologic stress. McFarlane et al. (1989) and McFarlane and Curtis (1989) evaluated consequences of multiple concurrent stressors on young chickens by hematologic parameters; however, hematologic information for wild birds is limited. Lisano and Kennamer (1977) profiled eastern wild turkey (*Meleagris gallopavo silvestris*) blood parameters in a descriptive study without induced treatment variables. Schulz et al. (2001) incorporated heterophil–lymphocyte (H:L) ratio as an indicator of stress in mourning doves (*Zenaida macoura*) but did not evaluate entire white blood cell (WBC) differentials or estimate WBC counts.

We incorporated quantification of avian hematologic parameters in our study to test for physiologic effects of radiotransmitter attachment methods by evaluating six different transmitter attachments as treatment types versus a control. Our experimental design overcame limitations of Schulz et al. (2001) by minimizing confinement of birds in limited space, incorporating broader hematologic measurements, and discriminating between gender and age classes.

MATERIALS AND METHODS

Two hundred ten white-winged doves caught in Kingsville, Texas, in grain-laden, wire funnel live traps were used (Reeves et al., 1968). Birds were acclimated to captivity for a minimum of 12 wk, and provided water ad libitum, and their diet was gradually converted to commercially prepared pelleted pigeon and dove feed (Purina Mills, Inc., St. Louis, Missouri, USA). Doves were handled in accordance with guidelines established by U.S. government's Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training and approved by Texas State University–San Marcos Animal Care and Use Committee, protocol 5QEKCT.

Birds were housed in 30 outdoor pens in three groups: adult males (nine pens), adult females (11 pens), and hatching year (HY) (10 pens), in sets of seven birds. Pens measured 1.83×1.83×1.83 m (6.11 m^3) and set 0.67 m above ground (Mirarchi, 1993). Alignment of pens consisted of back-to-back rows of 15 pens each. Males, females, and HY doves were segregated into pens containing one individual from each treatment. The gender of adult doves was identified by cloacal characteristics (Miller and Wagner, 1955). Treatments included an external transmitter attached with cyanoacrylate glue adhesive (ADH), double-loop body harness (HRN), surgical subcutaneous implant (SCI), surgical intracoelomic implant (ICI), subcutaneous surgery without implant (SCS), intracoelomic surgery without implant (ICS), and a control with no transmitters or surgeries applied (CNT). Because we used captive birds for this study, all transmitters were nonfunctioning, exact replicates of working transmitters (Zenitsky, 1993) and cost one-tenth as much as functional transmitters. Internal and external transmitter packages weighed 3.0 and 3.5 g, respectively. Implant transmitters were obtained from Advanced Telemetry Systems (ATS, Insanti, Minnesota) and sterilized before surgery by soaking in a 2% solution of chlorhexidine gluconate (Aspen Veterinary Resources, Kansas City, Missouri, USA) for 10 min. Surgeries were done in a laboratory by the protocol described by Small et al. (2004b). Transmitters and attachment methods are described in Small et al. (2004b).

Blood was collected by puncturing the medial metatarsal vein with a 25-gauge needle and drawing blood into heparinized microhematocrit tubes (Fisher Scientific, Pittsburgh, Pennsylvania, USA). Blood film smears were made using the push method protocol (Campbell, 1988). All smears were made by the same, experienced operator to minimize potential differences in blood smear thickness. Hemocytometry was not used because of the large number of samples. Blood smears, air dried, were fixed with 95% methanol and stained with modified Wright-Giemsa, using an Ames Hematek Slide Stainer (Miles Laboratory, Elkhart, Indiana, USA). The stain solution consisted of 0.72% w/v polychrome methylene blue-eosin, 99.28% v/v methanol, and the buffer contained 0.11% w/v KPO_4; 0.07% w/v $NaPO_4$; 7.5% methanol; 92.32% nonreactive ingredients and preservative (Romanowsky, 1981).

For each sampling date, duplicate slides were made for each dove. Blood samples consisted of six collections: one pretreatment and five posttreatment collections. Pretreatment sampling occurred on 18–19 August 1998, and posttreatment sampling on 30–31 August, 8–9 September, 8–9 October, 29–30 October, and 19–20 November 1998. All blood samples for

specific collection periods were drawn within 48 hours for a temporally discrete WBC profile for all birds.

All WBC counts and differentials were performed by the same, experienced person to minimize potential bias. Mean values of five random fields of view (FOV) were used to determine semiquantitative total WBC counts. The FOV means of WBC counts were multiplied by a correction factor of 2,000 in calculating a total WBC count for each sample for each sampling date for every bird. The FOV and differential counts were done on a Nikon E400 Eclipse light microscope (NikonUSA, Melville, New York, USA) at 500×, using immersion oil. Identity of granulocytes was confirmed at 1,000× oil immersion. Leukocyte types for WBC differentials were determined following blood cell morphology guidelines established by Campbell (1988) and Hawkey and Dennett (1989). Differentials were done by counting 100 WBCs and recording percentages for each type of WBC (heterophils, eosinophils, basophils, lymphocytes, and monocytes). Hematologic values were analyzed by multivariate analysis of variance (MANOVA) and mixed-model factorial repeated measures analysis of variance (ANOVA) on dependent variables: WBC count, WBC differential, and H:L ratios. Randomized block MANOVA is the suggested method for handling multiple dependent variables in an experimental design (Seal, 1964; Cooley and Lohnes, 1971; Digby and Kempton, 1987; Southwood and Henderson, 2000). We used Boneferroni post hoc criteria for a posteriori comparisons (Zar, 1988) at $\alpha \leq 0.05$ to show significance.

RESULTS

Multivariate analysis of variance (MANOVA) revealed significant differences among pens for dependent variables in this experiment (Wilk's Lambda=0.64, $P<0.05$). Univariate tests revealed significant differences among pens for WBC counts ($F_{20,694}=7.9$, $P<0.05$), heterophil counts ($F_{20,694}=1.9$, $P<0.05$), basophil counts ($F_{20,694}=2.2$, $P<0.05$), and monocyte counts ($F_{20,694}=1.7$, $P<0.05$). Differences among pens justified the use of pen as a blocking variable for examining differences among treatments and controls. With a pen as a blocking variable and gender nested, a randomized block MANOVA revealed no significant differences among six treatments and a control (Wilk's Lambda=0.96, $P>0.05$). Data used for the analysis are presented in Table 1.

Repeated measures ANOVA revealed significant differences across sampling periods for total WBC counts ($F_{5,220}=18.0$, $P<0.05$), heterophil counts ($F_{5,115}=2.6$, $P<0.05$), lymphocyte counts ($F_{5,115}=3.3$, $P<0.05$), H:L ratio ($F_{5,220}=5.0$, $P<0.05$), eosinophil counts ($F_{5,220}=4.8$, $P<0.05$), and monocyte counts ($F_{5,220}=14.6$, $P<0.05$). However, no significant differences in treatments within sampling periods occurred for any of the variables: total WBC counts ($F_{30,220}=1.0$, $P>0.05$), heterophil counts ($F_{30,115}=0.5$, $P>0.05$), lymphocyte counts ($F_{30,115}=0.3$, $P>0.05$), basophil counts ($F_{30,220}=1.0$, $P>0.05$), eosinophil counts ($F_{30,220}=0.6$, $P>0.05$), monocyte counts ($F_{5,220}=0.8$, $P>0.05$), and H:L ratio ($F_{30,220}=1.9$, $P>0.05$).

Significant differences across sampling dates for all treatments occurred for gender groups for H:L ratios, but not within gender and not within sample periods across treatments. Tabulations by gender are presented in Table 2. Total WBC counts increased from sample periods 1 through 3, with a subsequent decrease (Fig. 1). H:L ratios decreased sharply from sample periods 1 to 2 and then remained relatively stable (Fig. 2).

DISCUSSION

This is the first study addressing physiologic stress induced from radiotransmitter attachment using quantification of leukocytes in white-winged doves. On the basis of no difference in total or differential leukocyte counts or H:L ratios between treatments versus a control, there was no evidence that radiotransmitter attachment resulted in stress in this study. Our results are similar to those of Schulz et al. (1998, 2001), who found no evidence that transmitter attachment caused stress in mourning doves. However, the authors used H:L ratios exclusively and did not correlate stress with total WBC count and complete WBC differentials, nor did they account for change in total WBC count across all

TABLE 1. Descriptive profile, mean (standard error), for white blood cell differentials for captive white-winged doves with different radiotransmitters attachment packages from August to November 1998.

Treatment	White blood cell	Heterophils	Lymphocytes	Basophils	Eosinphils	Monocytes
Control	16,050.54 (1,302.7)	4,357.87 (477.1)	10,409.54 (1,097.6)	365.58 (34.3)	512.94 (70.2)	400.08 (87.5)
Subcutaneous Implant	16,926.81 (1,608.3)	4,654.15 (364.7)	10.836.46 (1,200.7)	395.03 (29.8)	496.76 (97.0)	442.27 (113.5)
Backpack	16,730.67 (1,699.6)	4,628.72 (640.3)	10,629.47 (1,130.7)	375.49 (32.7)	645.98 (94.8)	451.02 (103.7)
Intracoelemic Implant	15,513.96 (1,302.8)	4,666.84 (511.8)	9,478.71 (978.0)	347.38 (45.0)	580.70 (93.4)	437.16 (86.3)
Subcutaneous Surgery	16,299.96 (972.4)	4,450.05 (486.7)	10,605.28 (1,010.3)	409.95 (56.2)	456.72 (50.5	377.95 (81.6)
Glue-on	16,785.72 (1,494.3)	4,746.74 (441.0)	10,745.74 (1,148.6)	344.18 (22.1)	533.68 (89.2)	380.18 (84.6)
Intracoelemic Surgery	16,304.18 (1,400.7)	4,596.28 (371.6)	10,417.05 (1,119.8)	360.58 (55.8)	515.82 (87.1)	394.22 (77.4)

TABLE 2. Mean heterophil-lymphocyte ratios for captive white-winged doves with different radiotransmitter attachment packages from August to November 1998.

	Male						Female					
	Sampling period						Sampling period					
Treatment	Pre	1st Post	2nd Post	3rd Post	4th Post	5th Post	Pre	1st Post	2nd Post	3rd Post	4th Post	5th Post
Control	0.710	0.232	0.549	0.461	0.404	0.545	1.017	0.393	0.374	0.519	0.445	0.332
Subcutaneous Implant	0.759	0.345	0.429	0.296	0.623	0.740	0.911	0.280	0.544	0.350	0.498	0.370
Backpack	0.476	0.305	0.788	0.417	0.391	0.892	0.504	0.269	0.552	0.292	0.446	0.393
Intracoelemic Implant	0.537	0.381	0.567	0.296	0.576	0.826	1.910	0.245	0.509	0.523	0.493	0.381
Subcutaneous Surgery	0.452	0.187	0.577	0.383	0.628	0.788	1.404	0.478	0.467	0.316	0.374	0.435
Glue-on	0.512	0.356	0.727	0.448	0.571	0.747	1.204	0.476	0.447	0.288	0.317	0.382
Intracoelemic Surgery	0.624	0.201	0.472	0.479	0.438	0.899	1.225	0.448	0.443	0.321	0.508	0.334

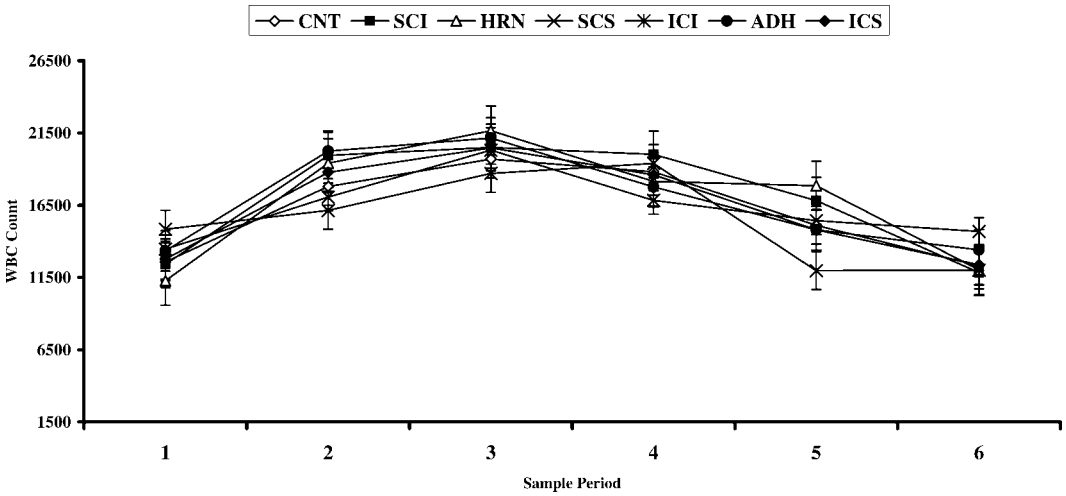

FIGURE 1. White blood cell counts (WBC) by sample period (1=pretreatment, 2, 3, 4, 5, 6=posttreatment) for captive white-winged doves with different radiotransmitter attachment treatments (CNT=control, SCI=subcutaneous implant, HRN=harness, SCS=subcutaneous surgery, ICI=intracoelemic implant, ADH=adhesive, ICS=intracoelemic surgery) in 1998.

treatments and across gender and age groups over time. Although increased WBC counts are primarily indicative of an inflammatory response and H:L ratios are excellent indicators of stress, knowing both values simultaneously may prove important. For example, H:L counts of 2:4 and 10:20 provide identical ratios but may be misinterpreted without comparison to total WBC differential values.

Changes in total WBC counts over time in this study probably were not the result of captivity because previous studies using captive white-winged doves showed no abnormal response to captivity based on physiological, behavioral, and productivity

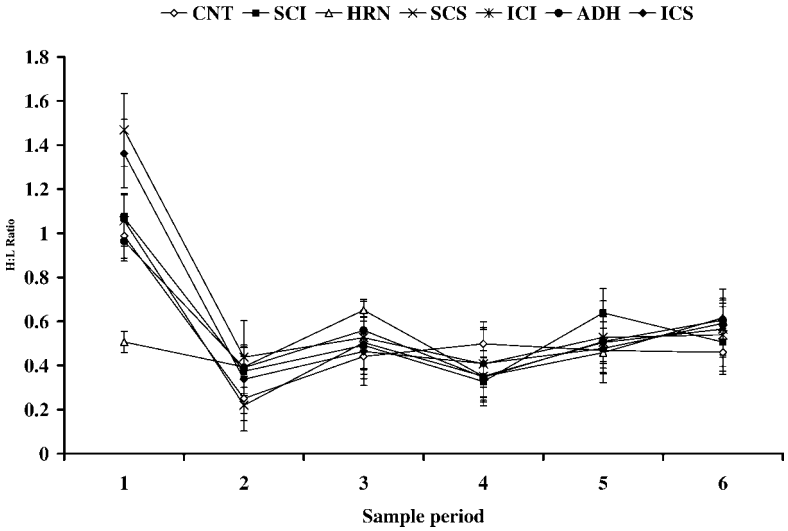

FIGURE 2. Heterophil-lymphocyte (H:L) ratios by sample period (1=pretreatment, 2, 3, 4, 5, 6=posttreatment) for captive white-winged doves with different radiotransmitter attachment treatments (CNT=control, SCI=subcutaneous implant, HRN=harness, SCS=subcutaneous surgery, ICI=intracoelemic implant, ADH=adhesive, ICS=intracoelemic surgery) in 1998.

analyses (Rosales, 2000; Small et al., 2004b). Because white-winged doves are traditionally migratory and our study period overlapped the beginning of the migration period, it is plausible that the fluctuation in WBC production was an adaptive response to pending migration. Migration in white-winged doves is directly linked to photoperiod (Wu et al., 2000) and the subsequent production of melatonin (the primary hormone influencing migratory behavior) from the pineal gland (Sturkie, 1976; Ritchie et al., 1994). In turn, increase in melatonin has been directly linked to increased production of WBCs (Klein et al., 1997; Whittow, 2000).

In our study, significant differences across sampling dates for all treatments occurred for both gender and age groups for WBC, lymphocyte, heterophil, eosinophil, and monocyte counts and H:L ratios; however, not within sample periods across treatments. We also found that lymphocyte differentials were more indicative of absolute WBC count than either heterophil or H:L ratios. Hematologic profile studies such as those described by Rosskopf and Woerpel (1982), Rosskopf et al. (1982), and Speer (1995), which focus on economically important pet birds, show significant differences between species, individuals (Rosskopf and Woerpel, 1981), age, gender, captivity, and distance traveled (Speer, 1995). These findings, combined with those of our study, indicate that all captive and field studies in birds should consider age, gender, reproductive status, feeding habits, and captivity as potentially important variables in assessing blood parameters.

The value of quantifying clinical effects of transmitter attachments on wild bird species cannot be underestimated. Radiotelemetry has become increasingly popular for studying wild birds, particularly because of advances in technology that have reduced transmitter weight and increased transmitter longevity (White and Garrott, 1990). A fundamental tenant of using radiotransmitters to study animals is that the transmitters do not alter the fitness or behavior of the individuals being studied (White and Garrott, 1990). We found no evidence that transmitter attachment was detrimental in white-winged doves, based on hematologic parameters. Migratory aspects, abundant urbanization and distribution of breeding populations, importance as a game species, mosaic life history, and apparent tolerance to transmitter attachment qualify this bird as a candidate for continued telemetry studies (Small et al., 2004b; George et al., 1994).

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