Interaction of p,p′ DDT with Histomoniasis in Bobwhites

by

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DDT has become ubiquitous in the environment and because of its lipophilic nature accumulates in fatty biological tissues. Various physiological effects of DDT on animals have been reported (DUSTMAN and STICKELL 1969), and specific attention has been devoted to eggshell thinning and reproductive failure in birds of prey, particularly piscivorous species (DEWITT 1955, 1956, DUSTMAN and STICKELL 1969, ENDERSON and BERGER 1970, RUDD and GRENELLY 1956, SMITH et al. 1969, TUCKER and HAEGEL 1970). Animals which are infected with disease causing organisms are subject to further problems imposed by environmental stresses, such as famine and inclement weather. Pesticides which act on animal physiological systems provide a stress factor, even in sublethal quantities, which may increase susceptibility to disease (MOORE 1967). Most toxiciological research has dealt with threshold levels of pesticides in a particular species, but the effect of pesticides on disease course and the conditioning effect of pesticides for infectious disease have received little emphasis (FRIEND and TRAINER 1970).

Histomoniasis (enterohepatitus, blackhead disease) is an infectious disease of gallinaceous birds caused by a protozoan, Histomonas meleagridis, introduced into a host in eggs of the nematode, Heterakis gallinarum (REID 1967, LUND 1969). Some species, such as turkeys, are very susceptible to histomoniasis, while others, such as chickens, are more resistent. Chickens usually are little disturbed, with a mild cecal involvement which passes in three or four weeks (LUND 1969). LUND and ELLIS (1967) reported that this disease is unimportant in Japanese quail (Coturnix coturnix japonica). The quail neither succumb nor void sufficient numbers of infectious stages of the parasite to be significant in spreading histomoniasis to more susceptible birds. LARSON and HANSEN (1966) found that the Japanese quail are susceptible to Heterakis gallinarum, but may not be susceptible to Histomonas meleagridis or the enterohepatitus caused by it.

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Japanese quail and bobwhites are more susceptible than chickens, with the bobwhite being slightly more susceptible than the Japanese quail. LUND and CHUTE (1970) showed that bobwhites were poor hosts for Heterakis whether Histomonas was present or not. However, KELLOGG and REID (1970) showed conclusively that bobwhites are capable of serving as a source of blackhead infection under experimental conditions. Fewer bobwhites than turkeys die from the disease; therefore more carriers survive.

RADHAKRISHNAN et al. (1972) found that white leghorn chickens became more susceptible to histomoniasis following oral administration of sublethal doses of p,p'-DDT. They found that larger doses of DDT resulted in greater residues in the brain and liver, but at the lowest level of DDT fed, cecae contained near maximum residues. Quantitative evaluation of pathognomonic lesions of histomoniasis correlated with DDT residues in cecal tissues.

Materials and Methods

Fifty-seven 21-week old, pen reared bobwhites (Colinus virginianus) were placed in wire cages and fed chicken starter mash (free of coccidiostats and antibiotics) and water ad libitum. These birds were divided into six groups and treated as indicated in Table 1.

Table I

Dosages of p,p'-DDT and Heterakis gallinarum eggs used in this experiment.

Group	No. of Birds	Dosage mg/kg p,p'-DDT[1]	Eggs[2]
I	7	0.0	+
II	8	0.01	+
III	9	0.1	+
IV	9	1.0	+
V	15	10.0	+
VI	6	10.0	-

1. mg p,p'-DDT/kg Body wt., administered. p,p'-DDT from Perrine Primate Lab., EPA, Perrine, Fla. 99.8% pure.

2. 1800 eggs of Heterakis gallinarum

The DDT was diluted in corn oil and administered orally via a tuberculin syringe without a needle. After 1 administration every other day for 10 days, the Heterakis eggs were given orally via a pipette. Following egg innoculation, 15 administrations of pesticide were given, 1 every other day. Eggs laid during the experiment were collected approximately every other day from five groups; no eggs were produced in Group V. Eggs were initially frozen, then refrigerated until analysis. They were then ground (shell included) and analyzed for p,p'-DDT, p,p'-DDD and p,p'-DDE. After 43 days the birds were sacrificed. The livers and cecae were scored on the basis of severity of lesions. Brain, liver and cecal residues (organs from each group were pooled and average group organ residues determined) were analyzed for p,p'-DDT, p,p'-DDD and p,p'-DDE. Birds that died during the course of the experiment were analyzed separately. Lesions were scored as follows:

Lesion Grades with Explanation (LUND 1969)

0 = Cecae and liver normal

\+ = Slight thickening and casseation of cecae, small or spherical liver lesions.

++ = Moderate thickening and casseation of cecae, larger liver lesions.

+++ = A necrotic cecal core seen, liver lesions very large, crater-like.

++++ = Cecal core very marked and liver lesions have concentric rings with elevated edges.

The cecal lesions in each of the two lobes of each bird were averaged, and a group average was calculated. The liver lesions were also averaged for each group.

Each sample (as well as a feed sample) was covered with an appropriate amount of anhydrous sodium sulfate and was ground in a blender until a free flowing powder was produced. This powder was packed into a 43 x 123 mm Whatman extraction thimble and extracted in a Soxhlet apparatus for 7 hours with petroleum ether. After evaporation of the solvent, the sample was taken up in 50 ml of acetonitrile saturated with hexane, partitioned with 50 ml of

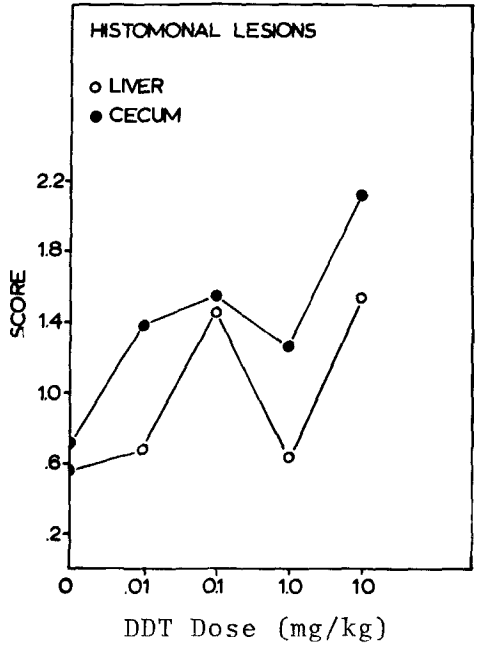

Figure 1

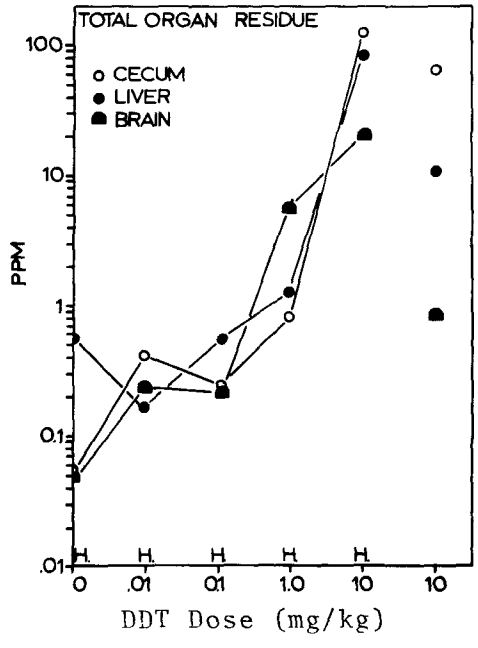

Figure 2

Figure 1. Severity of liver and cecal lesions in diseased birds fed p,p'-DDT ranging from 0 to 10 mg/kg body weight.

Figure 2. Total DDT (p,p'-DDE, p,p'-DDD and p,p'-DDT) recovered from brain, liver and cecum for all groups. Birds dying before day of sacrifice are included.

hexane saturated with acetonitrile and washed three times with 50 ml portions of hexane to separate pesticide residues from tissue lipids. The acetonitrile was evaporated and lipid weights were determined. To remove remaining lipids, the extract was placed on a florisil column containing 8.2% water deactivated florisil via three 15-ml hexane rinses. The column was eluted with ~200 ml hexane: benzene (3:1). One ul of this eluate, after being appropriately concentrated or diluted, was injected into a Varian 2100 gas Chromatograph containing a 1.6% OV 17/6.4% OV 210 on 60/80 mesh Chromosorb W in a 6' x 1/4" glass column with an electron capture detector. Operating temperatures were: injection port, 210°; column, 200°; and detector, 210°. Nitrogen flow rate was 50 ml/minute. Percent recovery using this method was 80-100%.

Results and Discussion

As shown in Figure 1, both cecae and liver lesions were more pronounced in Group V (10 mg, p,p'-DDT/kg body wt + 1800 H. gallinarum eggs). A sharp decrease in lesion severity occurred in Group IV. Percent mortality in Groups V and IV was 26.6% and 25.0% respectively. There were no deaths in Group III, and 11.1% mortality occurred in Group II. In the two control Groups, I and VI, no birds died as a result of histomoniasis.

In bobwhite, LUND (1969) observed that cecal cores were not seen until the third week after infection, and liver involvement was infrequent. In this experiment morbidity and mortality were evident after 11 days. Of seven birds determined to have died as a result of experimental treatment, 5 were male. This is in agreement with the report of GISH & CHURA (1970) who found that female birds (Japanese quail) in breeding condition have a survival advantage over other birds.

Figure 2 shows that residues increased with increased levels fed and that organ residues did not reach an appreciable amount until 10 mg DDT/kg body weight was administered. Controls fed no pesticide had residues because the chicken starter mesh diet contained residues of 0.01 ppm total DDT. At the 10 mg/kg level diseased birds appeared to retain greater amounts of DDT than non-diseased birds.

Egg collection began on the first day of the experiment. Figure 3 illustrates the production of eggs throughout the experimental period. It can be seen that those birds fed the lesser amounts of

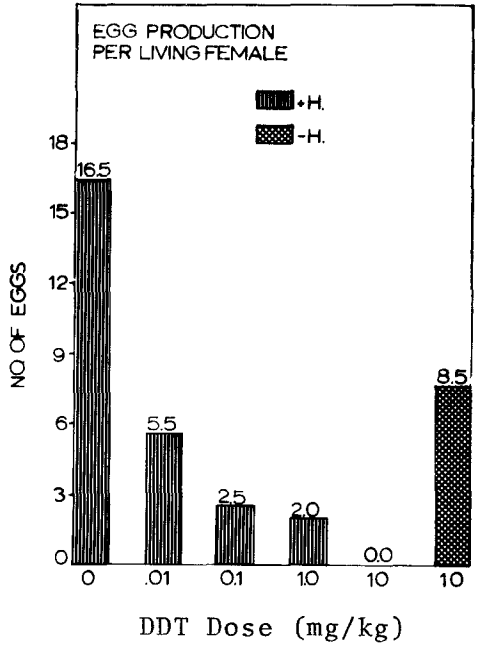

Figure 3. Egg production for all groups per living female. Eggs collected every other day and divided by number of living females. +H = Heterakis eggs administered; -H = no Heterakis eggs administered. Birds receiving 10 mg/kg dose producing no eggs received Heterakis eggs.

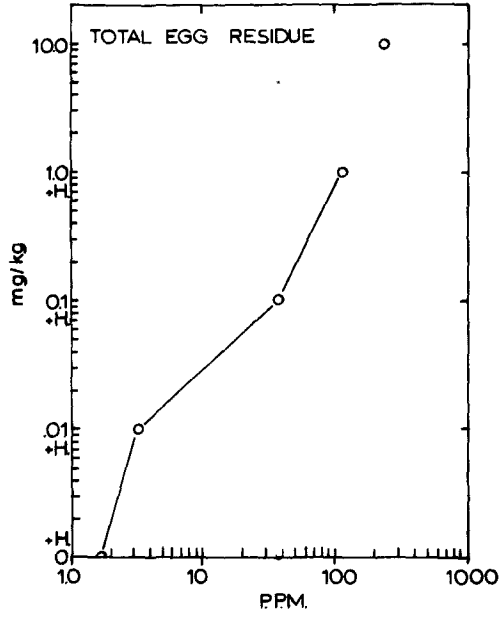

Figure 4

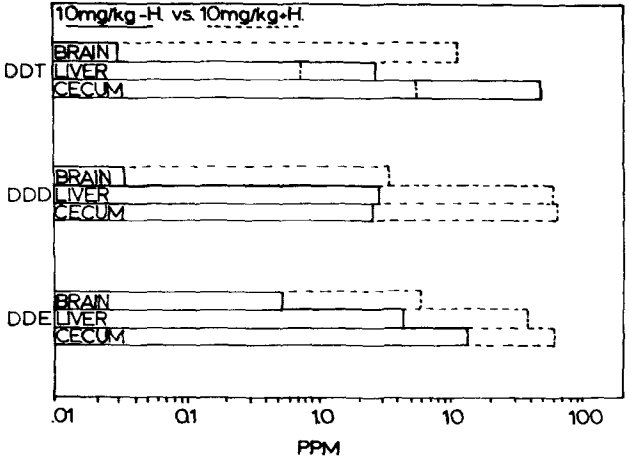

Figure 5

Figure 4. Comparison of p,p'-DDE, p,p'-DDD and p,p'-DDT found in brain, liver and cecum of 10 mg/kg + disease (+H) group with the 10 mg/kg control (-H) group.

Figure 5. Total DDT in eggs recovered from all groups.

pesticide and given H. gallinarum eggs laid more eggs. Group VI (10 mg DDT/kg body wt + no H. gallinarum eggs) produced fewer eggs than Group I (no pesticide + 1800 H. gallinarum eggs) and more than Group II (.01 mg DDT/kg body wt + 1800 H. gallinarum eggs). Group V (10 DDT/kg body wt + 1800 H. gallinarum eggs) produced no eggs.

Pooled DDE, DDD and DDT residues in brain, liver and cecum in the groups fed 10 mg/kg, one with the disease organisms, the other without, are shown in Figure 4. The total residue in the birds with disease was greater than in those without the disease. There also appears to be a shift in the metabolic pattern of DDT in the diseased birds. In diseased birds more DDT concentrated in the brain but less was found in liver and cecae.

Excluding the non-diseased group, correlation coefficients of DDT level fed as ppm versus brain and cecal DDE, DDD and DDT residues ranged from .917 to .996; liver coefficients were .999 for DDE, .996 for DDD and -.217 for DDT. Disease severity, as measured by cecal and liver lesions, was compared with DDT level fed and respective organ residues. Coefficients for DDE, DDD and DDT ranged from .790 to .804 in cecae, and .649 to .666 for DDE and DDD, and -.446 for DDT in liver.

Figure 5 illustrates that total DDT residue in eggs increased in proportion to the doses fed. Because the 10 mg/kg DDT group without disease produced more eggs than any of the DDT plus disease groups (Fig. 3), the decrease in egg production from the lower DDT groups to the higher ones cannot be attributed solely to the insecticide fed. What is illustrated is that lowered egg production in this study resulted from the combined effect of histomoniasis and increased DDT levels in bobwhites. The insecticide DDT rendered bobwhites more susceptible to histomoniasis, which under normal environmental conditions, they would not have contracted as quickly or as severely.

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