EFFECTS OF MODERATE ASCARID INFECTIONS UPON THE RESISTANCE OF CHICKENS TO BACTERIAL TOXIN

by

DEAN SYDNEY FOLSE

B. S., Agricultural and Mechanical College of Texas, 1945
D. V. M., Agricultural and Mechanical College of Texas, 1945

A THESIS

submitted in partial fulfillment of the

requirements for the degree of

MASTER OF SCIENCE

Department of Zoology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1946

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INTRODUCTION

Large numbers of ailing chickens are sent each year by producers to commercial and animal health laboratories for diagnosis. Upon post-mortem examination these fowls frequently show only moderate worm infections.

Heavy infections of the fowl nematode Ascaridia galli (Schrank) have been shown by Ackert and Herrick (1928) to produce in growing chickens such symptoms as sluggishness, loss of appetite, ruffled feathers, drooping wings, loss of blood and body weight, retarded muscle and bone development, and increased mortality. Other effects of large infections may be reduction in blood sugar level (Ackert and Titus, 1924) and shrunken thymus glands (Ackert, 1930).

On the other hand, Ackert and Wisseman (1946) have obtained results which indicate that growing chickens may tolerate moderate infections of ascarids and tapeworms if the fowls are under proper management and on a completely adequate diet.

This raised the question of the importance of moderate infections of fowl nematodes. It seemed logical that even moderate infections might have some intrinsic effect on chickens. It was to test this hypothesis that the present study was undertaken; namely, to ascertain whether a moderate infection of Ascaridia galli would predispose chickens to the effects of a bacterial toxin.

REVIEW OF LITERATURE

Ackert and Herrick (1928) pointed out that most severe symptoms of heavy parasitism with the fowl ascarid are seen during the first three weeks of parasitism. Causes of these symptoms may be intestinal injury, loss of blood, and bacterial infection or absorption of waste products excreted by the worms. Baker, Conklin, et al. (1929) and Cram (1930) observed similar symptoms in heavy ascarid infections.

More obscure symptoms are reduction in the size of the thymus gland (Ackert, 1930) and reduction in the sugar content of the blood (Ackert and Titus, 1924). Nematodes may also make possible other infections in poultry such as bacterial invasion of the intestine (Cram, 1930), but experimental proof of such invasion appears to be lacking.

It has been pointed out that growing birds given an adequate balanced ration, apparently can tolerate moderate numbers of ascarids with no outward manifestations. In controlled experiments comparisons of parasitized and nonparasitized birds showed no significant difference in body weight, percent of hemoglobin, or blood sugar levels (Ackert and Wisseman, 1946). It has been shown, however, that there is a definite drop in the growth curve of chicken weights 10 to 14 days after feeding chickens moderate numbers of worm eggs (Riedel, 1946).

Botulism in chickens has been known in the United States since 1917 when it was first considered as a possible cause of

"limberneck" by Dickson (1917) who ascribed to it symptoms of sluggishness and weakness. Wilkins and Dutcher (1920) concluded that the term "limberneck" is a symptom rather than a disease. The principal clinical symptoms as reported by Hart (1920) are ruffled feathers, paralysis, inability to walk, wings resting on the ground, eyes dull, refusal to eat, coma, and death. It was soon found that Botulinus toxin (Type A) caused the same symptoms in chickens as those caused by Type C (Graham and Schwarze, 1921).

MATERIALS AND METHODS

Day old Single-Comb White Leghorn chicks were obtained from a commercial hatchery in Wichita, Kansas. They were placed in an electrically heated brooder and kept constantly over wire cloth for one month. An adequate ration, as prescribed by poultrymen, and clean water were kept before them constantly except at times of handling, when they were removed for only a few minutes.

At four weeks of age the chicks were weighed, banded with consecutively numbered wing bands and divided into three main groups. After all weights had been recorded, the birds that were exceedingly heavy or very light were culled out. A representative weight was arbitrarily taken and if three birds of this weight were found, each was placed in a separate group (I, II, or III). When three birds of exactly the same weight could

not be found, approximate equals were used, and in the next selection differences were equalized within the group.

Group I consisted of parasitized and injected chickens, Group II of injected chickens not parasitized, and Group III of control chickens neither parasitized nor injected.

The parasite eggs were obtained from worms (Ascaridia galli) taken from recently killed chickens at a commercial poultry plant. The worms were washed in tap water and then poured into a bowl of distilled water. Mature female worms were taken singly from the bowl, put into a clean Petri dish, and opened by having the anterior end of the worm excised with a scalpel. The contents of the worm were expressed by holding the posterior end of the worm with forceps and applying pressure with a spatula from the posterior end progressively forward, after which the ovaries and intestine were teased away from the uteri containing the eggs. The uteri were then washed in distilled water and placed in a sterile covered Petri dish. The eggs were examined under a microscope and only uteri containing fertilized eggs were saved. When four or five pairs of uteri had been separated and placed in the Petri dish, they were mashed with the spatula and the eggs evenly distributed in the dish. The date, number of uteri contained, and the worker's initials were put on the outside of each dish. The eggs were allowed to dry five minutes before distilled water was added slowly at the edge of the culture until it was about 6.0 mm deep

in the dish. About ten drops of two percent formalin solution were then added and mixed with the water. The culture was placed in an incubator and held at 30° C. until used; the water and formalin solution was changed approximately every other day.

The birds in Group I were parasitized by the drop method of Riedel (1946) as soon as separated and placed together in one battery unit. The eggs of the cultures were removed by scraping the bottom of the Petri dishes with the edge of a spatula and washing the free eggs out with as small an amount of distilled water as possible, placing the eggs in a glass dropper bottle. A few grams of clean fine sand were added to the bottle and the mixture shaken well to separate the eggs and disperse them in the water. Drops of the egg suspension were put on several slides singly and the embryonated eggs in each were counted. By determining the mean number of infective eggs in each drop the dosage of worms to a bird could be regulated by the number of drops given. The chicks in Group I were taken singly and held by placing the birds' feet under the worker's left arm. The chick, on its back, was held by the head with the left forefinger in one commissure of the beak and the left thumb in the other. Two drops containing approximately 150 embryonated eggs each were then put into the opened mouth, care being taken to mix the suspension just before dosing.

Botulinus toxin (Type A) was selected because it is not infective, is easily obtained, and is standardized. One milligram of the dried toxin, obtained from the National Institute of

Health in Washington, D. C., was weighed on an analytical balance. This was put into 10 cc of sterile saline solution and thoroughly dissolved; 1.0 cc of this solution was added to each 9.0 cc of sterile saline used and this was thoroughly mixed.

On the 14th day after parasitizing Group I, the chickens in Groups I and II were injected intraperitoneally with this toxin solution, at the rate of 0.0002 mg/Kg of body weight in Experiment 1 and 0.0001 mg/Kg in Experiment 2. To inject the toxin, the bird was held by placing its head under the worker's left arm and with the left hand grasping the skin just behind the sternum. The skin was disinfected with 70 percent isopropyl alcohol and then held taut so that the needle could be inserted into the peritoneal cavity.

Periodic observations were made from the time of injection until the termination of the experiment. Record of the number of chickens that were weak, unable to rise, or dead was taken at each observation. In Experiment 1 only the number of birds was recorded, but in Experiment 2 the wing band number of each ailing bird was also recorded.

The worms were recovered from the dead birds or at the end of the experiment by flushing the intestines with warm water under pressure (method of Ackert and Nolf, 1929). All injected chickens developed diarrhea, and those that died during the course of the experiment showed severe hemorrhagic enteritis.

DATA

Experiment 1

The dose of toxin used in this experiment for Group I and Group II was 0.0002 mg/Kg of chicken body weight. The incubation period of the toxin was 11 hours, which is shorter than that given by Hart (1920). Group III (controls) was not parasitized and not injected.

The first symptom seen was a decrease in irritability, which was evident as early as four hours after injection. By the 11th hour, 12 Group I birds were noticeably weak, 11 of them being unable to rise; only three Group II birds were weak but none of them was unable to rise. Twelve hours later 17 in Group I were weak, but none in Group II (Table 2).

Twenty-five hours after injection 14 chickens in Group I were unable to rise, as compared with six in Group II. The first death was in Group I, occurring at 30 hours after injection. There were 14 birds in Group I still unable to rise and eight in Group II. Two more Group I birds died at 48 hours with the first Group II death occurring at this time. The number of birds in Groups I and II unable to rise at this time was 11 and seven, respectively.

At 50 hours, seven Group I chickens were down and one died, while nine were down in Group II and none died. By the 54th hour there had been five deaths in Group I with 12 of the

remaining birds unable to rise; in Group II there had been two deaths with 13 of the remaining ones unable to rise.

By the 72nd hour after injection seven more Group I chickens died bringing the total dead to 13. Group II had by this time had only four deaths. From the 72nd hour until the termination of the experiment, a few birds in each of Groups I and II died intermittently. The later deaths were probably due to secondary causes that originated from the toxin but were not directly due to it. The number of deaths in Group II came within one of equaling the number in Group I by the time the experiment was terminated.

The average number of worms per chicken in Group I (parasitized and injected) was 2.84, with the highest being 12 in two birds; 10 of the chickens were without worms. Group II was free from worms. The small number of worms recovered may have been due to the diarrhea which the toxin produced.

Examination of the weights of the chickens as shown in Table 1 showed wide variations and an indication of an uncontrolled factor in Group III (unparasitized and uninjected) during the course of the experiment. At the second and third weighings their gains were notably less than those of the injected chickens. At the close of Experiment 1 the differences of average weights of Group I, II, and III were within the range of experimental error.

The results of this experiment in which 0.0002 mg/Kg of body weight of Botulinus toxin (Type A) was injected into two

Table 1. Comparative data of Experiment 1, including: chicken numbers, weekly weights, amount of Botulinus toxin solution injected, hours lived after injection, and number of worms recovered. (Parasitized November 20, 1945; injected December 4, 1945; and experiment terminated December 11, 1945.)

Group I								Group II								Group III						
Chicken number	Weight (gm) 11/20	11/27	12/4	12/11	Toxin solut. (cc)	Hours lived after injec.	No. of worms recov.	Chicken number	Weight (gm) 11/20	11/27	12/4	12/11	Toxin solut. (cc)	Hours lived after injec.	No. of worms recov.	Chicken number	Weight (gm) 11/20	11/27	12/4	12/11	Toxin solut. (cc)	Hours lived after injec.	No. of worms recov.
A2985	181	263	368	-	0.65	54	10	A2992	181	272	410	-	0.80	72	0	A3014	200	278	374	479			0
A2986	226	318	450	412	0.85	176	4	A2995	227	301	412	-	0.80	129	0	A3016	191	266	-	-			0
A2987	170	220	307	-	0.60	48	7	A2999	202	290	422	-	0.82	52	0	A3019	221	298	-	-			0
A2988	210	298	442	-	0.85	30	12	A3005	212	330	464	-	0.86	96	0	A3021	152	216	-	-			0
A2989	234	358	520	-	1.05	96	0	A3006	180	260	384	-	0.68	96	0	A3022	208	310	-	-			0
A2990	218	324	443	-	0.84	84	0	A3009	190	274	483	-	0.88	84	0	A3023	203	276	400	522			0
A2991	192	283	421	-	0.82	84	1	A3010	150	228	348	404	0.65	176	0	A3026	197	256	366	454			0
A2993	160	243	360	-	0.66	132	0	A3012	200	289	414	-	0.81	57	0	A3029	182	263	-	-			0
A2996	152	236	343	-	0.64	72	1	A3017	240	324	453	-	0.85	144	0	A3034	230	319	-	-			0
A2997	167	236	310	-	0.61	72	0	A3018	220	303	430	-	0.83	84	0	A3037	260	344	-	-			0
A2998	200	262	368	-	0.66	72	0	A3024	167	234	338	-	0.63	96	0	A3038	238	305	430	526			0
A3000	200	295	414	-	0.81	72	0	A3028	237	311	457	-	0.85	96	0	A3040	222	293	416	548			0
A3001	177	292	394	499	0.79	176	0	A3031	222	282	416	-	0.81	86	0	A3045	184	251	-	-			0
A3003	240	360	473	576	0.87	176	1	A3035	194	243	344	-	0.65	96	0	A3047	204	284	440	542			0
A3004	223	319	442	548	0.85	176	2	A3042	230	350	548	680	1.05	176	0	A3048	238	329	484	588			0
A3007	208	310	474	-	0.88	48	0	A3049	254	323	432	-	0.83	96	0	A3050	222	310	432	541			0
A3013	219	287	408	512	0.80	176	6	A3056	162	192	256	287	0.45	176	0	A3052	194	278	412	540			0
A3015	250	310	425	-	0.83	96	0	A3059	317	444	604	-	1.20	48	0	A3055	199	282	366	500			0
A3025	199	270	381	-	0.68	50	3	A3062	190	248	318	368	0.61	176	0	A3057	165	244	340	546			0
A3030	240	323	445	546	0.84	176	1	A3063	220	314	454	580	0.85	176	0	A3058	230	325	470	618			0
A3041	206	281	405	-	0.80	72	2	A3064	242	356	508	520	1.00	176	0	A3060	310	196	284	385			0
A3046	188	264	364	-	0.66	72	8	A3071	164	227	330	-	0.63	84	0	A3066	182	263	367	450			0
A3051	194	270	400	-	0.80	72	0	A3072	191	444	612	-	1.21	96	0	A3068	181	270	389	474			0
A3053	308	426	568	-	1.05	54	1	A3073	197	252	348	-	0.65	96	0	A3070	181	256	368	431			0
A3069	220	292	399	-	0.80	96	12	A3075	206	292	436	558	0.83	176	0	A3074	211	296	392	484			0
Average	207	293	413	515	0.78	97	2.8		207	295	424	483	0.81	113	0		208	280	373	507			0

Table 2. Comparative records of occurrence of symptoms of botulism in the groups of chickens in Experiment 1 including hours after injection, and number of chickens weak, unable to rise, or dead.

Hours after injection	Group I			Group II			Group III		
	Weakness	Inability to rise	Death	Weakness	Inability to rise	Death	Weakness	Inability to rise	Death
11	12	11		3					
23	17								
25		14			6				
30		14	1		8				
48		11	2		7	1			
50		7	1		9				
52		14			14	1			
54		12	2		13				
57						1			
72			7			1			
84			2			3			
86						1			
96			3			8			
129						1			
132			1						
144						1			
176			6			7			

groups of chickens showed that the parasitized group manifested symptoms first and had a higher mortality during the early part of the experiment than did the unparasitized chickens. Group III (unparasitized and uninjected) showed no symptoms.

Experiment 2

In Experiment 2 the dose of toxin was reduced to 0.0001 mg/Kg of fowl body weight. Wing band numbers were taken of the chickens showing symptoms at each observation, but no other changes were made from the method in Experiment 1. The incubation period conformed more to the typical period, being about 48 hours.

The first symptom was seen at 43 hours after injection, in a Group I chicken. By the 47th hour this bird was unable to rise and in eight more hours was dead. Sixty-seven hours after injection there were four Group I chickens unable to rise, but none in Group II. Two Group II birds were unable to rise at 71 hours as compared with five in Group I (Table 4).

Eight hours later the numbers of birds unable to rise were eight in Group I and three in Group II. Ten hours later, or 79 hours after injection, seven in Group I and the same three in Group II were unable to rise. By the 103rd hour all but one of the Group II birds were able to get up while seven of the Group I chickens could not rise.

Gradually, five of the seven in Group I that had been down

recovered; the other two died 139 hours after injection. There were no deaths in Group II (injected, unparasitized); and Group III (unparasitized and uninjected) showed no symptoms.

The average number of worms recovered from the Group I chickens was 0.92 worms. This small number of worms as before was probably due to the diarrhea caused by the toxin. The three chickens that died were found to have two, two, and zero worms, respectively.

As in Experiment 1, all groups had approximately the same average weight per chicken when parasitized. After the first week of parasitism (Table 3) Group I (parasitized and injected) averaged about 16 gm in weight less than Group II (unparasitized and injected) or Group III (unparasitized and uninjected). This lowered average may have been due to the worms.

In the second week after parasitism (time of injection) Groups I and II had approximately the same average weight per chicken and Group III averaged about 10 gm higher. At the termination of the experiment the Group I chickens averaged 135 gm less than Group II and 175 gm less than the chickens of Group III.

The results of this experiment in which 0.0001 mg/Kg of body weight of Botulinus toxin (Type A) was injected into two groups of chickens showed that the parasitized birds showed symptoms first, more of them became sick, and there was a definite increase in mortality in comparison to the unparasitized group which had only a few sick birds and no deaths.

Table 3. Comparative data of Experiment 2, including: chicken numbers, weekly weights, amount of Botulinus toxin solution injected, hours lived after injection, and number of worms recovered. (Parasitized February 28, 1946; injected March 14, 1946; and experiment terminated March 21, 1946.)

Group I								Group II							Group III						
Chicken number	Weight (gm) 2/28	3/7	3/14	3/21	Toxin solut. (cc)	Hours lived after injec.	No. of worms recov.	Chicken number	Weight (gm) 2/28	3/7	3/14	3/21	Toxin solut. (cc)	Hours lived after injec	No. of worms recov.	Chicken number	Weight (gm) 2/28	3/7	3/14	3/21	Toxin solut. (cc)	Hours lived after injec.	No. of worms recov.
100	304	374	510	438	0.51	170	1	A3109	310	406	532	664	0.53	170	0	A3112	310	396	516	644			0
101	268	362	480	442	0.48	170	0	A3117	270	348	446	540	0.45	170	0	A3115	382	528	618	758			0
102	310	390	538	480	0.54	170	0	A3121	348	492	584	720	0.58	170	0	A3120	346	394	524	662			0
103	252	344	456	-	0.46	139	2	A3125	258	340	430	480	0.43	170	0	A3124	316	418	556	708			0
104	280	360	490	469	0.49	170	0	A3130	264	380	496	582	0.50	170	0	A3132	264	334	494	630			0
107	270	352	468	456	0.47	170	0	A3133	268	370	464	594	0.46	170	0	A3138	268	332	434	526			0
110	278	380	482	380	0.48	170	0	A3134	296	420	516	670	0.52	170	0	A3143	350	390	528	636			0
111	350	440	624	550	0.62	170	0	A3137	230	312	420	484	0.42	170	0	A3146	296	358	416	-			0
113	312	390	538	480	0.54	170	0	A3140	312	426	544	566	0.54	170	0	A3155	254	332	468	582			0
114	348	380	570	-	0.57	139	2	A3144	306	410	520	612	0.52	170	0	A3160	360	498	634	760			0
116	266	350	440	414	0.44	170	0	A3145	354	452	584	636	0.58	170	0	A3161	270	378	482	618			0
119	254	338	406	340	0.41	170	6	A3148	250	322	428	544	0.43	170	0	A3162	230	328	442	580			0
122	230	308	404	370	0.40	170	0	A3151	350	450	570	666	0.57	170	0	A3168	294	424	558	726			0
123	332	440	560	604	0.56	170	0	A3152	254	348	436	528	0.44	170	0	A3170	254	320	440	548			0
126	340	434	550	516	0.55	170	2	A3153	282	374	476	550	0.48	170	0	A3176	280	382	514	638			0
129	296	366	504	480	0.50	170	0	A3158	278	370	380	464	0.38	170	0	A3180	344	444	532	582			0
135	356	456	570	547	0.57	170	1	A3163	240	340	444	564	0.44	170	0	A3181	260	348	456	560			0
136	240	346	448	432	0.45	170	0	A3164	224	316	400	520	0.40	170	0	A3182	220	294	382	491			0
139	220	308	420	382	0.42	170	3	A3165	300	444	560	644	0.56	170	0	A3184	294	400	510	620			0
147	300	416	550	496	0.55	170	1	A3172	340	454	546	680	0.55	170	0	A3185	304	470	640	828			0
150	300	400	510	494	0.51	170	0	A3173	330	420	530	596	0.53	170	0	A3186	250	344	444	550			0
154	292	400	480	453	0.48	170	1	A3174	258	368	454	574	0.45	170	0	A3188	294	416	510	570			0
166	230	306	394	-	0.39	55	0	A3178	230	326	436	544	0.44	170	0	A3189	238	346	476	636			0
167	258	340	448	470	0.45	170	3	A3179	304	412	550	648	0.55	170	0	A3190	230	316	420	532			0
169	254	364	480	446	0.48	170	1	A3183	294	428	574	666	0.57	170	0	A3191	332	460	612	770			0
171	270	350	480	350	0.48	170	0	A3187	272	376	504	636	0.50	170	0	A3193	268	384	490	630			0
Average	285	372	492	456	0.49	163	0.88		285	388	493	591	0.49	170			288	385	503	631			0

Table 4. Comparative records of occurrence of symptoms of botulism in the groups of chickens in Experiment 2 including hours after injection, and number of chickens weak, unable to rise, or dead.

Hours after injection	Group I			Group II			Group III		
	Weakness	Inability to rise	Death	Weakness	Inability to rise	Death	Weakness	Inability to rise	Death
43	1								
47		1							
55			1						
67		4							
71		5			2				
79		8			3				
91		5			3				
99		7			3				
103		7			1				
115		4							
123		3							
139		1	2						
143		1							
151	1								

DISCUSSION

The idea is commonly held among health of animal workers that infections of parasites predispose animals to bacterial and other infections. Statements that helminths lower the resistance of animals to bacterial infections are made in most textbooks of veterinary parasitology. In reviewing the literature of this subject no record of experimental evidence of nematodes lowering the host resistance to bacterial infection was found.

Defense of the fowl body against bacterial infections (toxins) may be made difficult by the ascaridia larvae being partially buried between the intestinal villi from the 10th to the 17th day of parasitism (Ackert, 1923). Ackert and Wisseman (1944) pointed out that while comparisons of averages were very similar, comparisons of individual parasitized chickens with their controls showed wide variations. They concluded that under conditions of good management and an adequate ration, chickens may tolerate moderate infections of Ascaridia galli without showing harmful effects.

Experimental evidence is here presented, apparently for the first time, to show that moderate ascarid infections may lower the resistance of chickens to bacterial toxin. This lowered resistance was demonstrated in the larger number of sick birds and the higher mortality rate in the parasitized groups than in the injected unparasitized ones.

SUMMARY

1. Two experiments were performed on 153 chickens to ascertain whether moderate infections of the fowl nematode, Ascaridia galli (Schrank), would predispose chickens to bacterial toxin.

2. The chickens which were given an adequate ration were divided into three groups by weight when they were one month old: Group I, parasitized, and injected with a Botulinus toxin; Group II, unparasitized, but injected; and Group III controls, unparasitized and uninjected.

3. Chickens were parasitized at one month of age by giving them approximately 300 embryonated eggs of A. galli.

4. The dosage of Botulinus toxin (Type A) was 0.0002 mg/Kg of body weight of chicken in Experiment 1 and 0.0001 mg/Kg of fowl body weight in Experiment 2.

5. Periodic observations were made from the time of injection until the experiments were terminated.

6. Criteria for comparing the effects of the toxin were: weakness, as manifested by reluctance of the fowl to rise; inability to rise; and death.

7. Worms were recovered by flushing the fowl intestines with warm water under pressure.

8. Group I had significantly more ailing birds than Group II, and a higher mortality in the early part of Experiment 1. In Experiment 2, Group I had significantly more ailing birds

than Group II, and three deaths as compared to none in Group II. Group III (unparasitized and uninjected) showed no toxic symptoms in either experiment.

Experimental evidence is thus presented, apparently for the first time, which shows that moderate infections of the fowl nematode, A. galli, may predispose chickens to the effects of a bacterial toxin.

ACKNOWLEDGMENTS

Acknowledgment is made to Dr. James E. Ackert, Professor of Zoology and Agricultural Experiment Station Parasitologist, for his guidance in the research and in preparation of this manuscript; to Dr. Donald J. Ameel, Head of the Zoology Department, for his counsel on the subject; to Professor V. D. Foltz, Department of Bacteriology, for his assistance in the preparation of the toxin solutions; and to Dr. H. C. Fryer, Department of Mathematics, for counsel on statistical treatment of data.

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