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EXPERIMENTAL TOXOPLASMOSIS IN PHEASANTS (*PHASIANUS COLCHICUS*)

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ABSTRACT: Sixteen 6-mo-old battery-reared ring-necked pheasants (*Phasianus colchicus*) were inoculated orally with 10^5 (group A, ME 49 strain, five birds), 10^4 (group B, ME 49 strain, six birds) and 10^4 (group C, GT-1 strain, five birds) *Toxoplasma gondii* oocysts. The pheasants in groups A and B remained clinically normal. One of the pheasants in group C died 19 days after inoculation (DAI); *T. gondii* was found in histological sections of brain and heart and encephalitis, myocarditis and enteritis were the main lesions. *Toxoplasma gondii* was isolated by bioassays from pooled tissues of five of six pheasants in group B killed 36 DAI. *Toxoplasma gondii* was isolated from the brains, hearts and skeletal muscles of each of the four pheasants inoculated with the GT-1 strain (group C), and from the brains of four, hearts of three and skeletal muscles of four of five pheasants inoculated with the ME 49 strain (group A). All pheasants developed high (1:1,600–1:25,600) antibody titers to *T. gondii* in the modified agglutination test (MAT) 36 to 68 DAI. Antibody titers detected with the MAT were higher than those detected in the indirect hemagglutination and latex agglutination tests. Antibodies were not detected in 1:4 dilution of pheasant sera with the Sabin-Feldman dye test.

Key words: *Toxoplasma gondii*, oocysts, tissue cysts, pheasants, *Phasianus colchicus*, antibody titers, agglutination tests, dye test.

INTRODUCTION

Many species of mammals and birds can be intermediate hosts of *Toxoplasma gondii* (Dubey and Beattie, 1988). *Toxoplasma gondii* infection is subclinical in many avian species, especially gallinaceous birds (Siim et al., 1963; Jacobs and Melton, 1966; Parenti et al., 1986; Dhillon et al., 1982; Hubbard et al., 1986; Biancifiori et al., 1986; Dubey and Beattie, 1988). Prevalence of *T. gondii* in avian species in the United States is largely unknown. Little is known concerning toxoplasmosis in pheasants. Recently Literák et al. (1992) isolated *T. gondii* from six of 306 pheasants (*Phasianus colchicus*) in Czechoslovakia. Antibody prevalence studies are unreliable if the Sabin-Feldman dye test or one of the non-reactive tests had been used. Our objectives were to study the serological responses of ring-necked pheasants (*Phasianus colchicus*) to experimental *T. gondii* infection and to compare four serological tests.

MATERIAL AND METHODS

Sixteen battery-reared 6-mo-old pheasants (12 females, four males) weighing 0.9 to 1.7 kg were used. The birds were divided into three groups; A, B, and C. Five birds (numbers 1 to 5) in group A, and six birds (numbers 6 to 11) in group B were inoculated orally with 10^5 (group A) or 10^4 (group B) infective oocysts of the ME 49 strain of *T. gondii*. The ME 49 strain of *T. gondii*, a strain of sheep origin, produces many tissue cysts in the brain of mice and is of mild virulence in mice (Lunde and Jacobs, 1983). Five birds (numbers 12 to 16) in group C each were given 10^4 infective oocysts of the GT-1 strain of *T. gondii*. The GT-1 strain of *T. gondii*, a strain of goat origin, is highly virulent in mice and one *T. gondii* of any developmental stage is lethal for mice (Dubey, 1980).

The oocysts were deposited directly in the crop by a canula. Oocysts had been obtained from the feces of experimentally inoculated cats, sporulated in 2% sulfuric acid, and stored for 11 mo (ME 49) and 2 mo (GT-1) at 4 C before use in the experiment. The number of infective oocysts in the inocula was determined by bioassays in mice (Dubey and Beattie, 1988). Inoculated birds were housed in sterilized wire cages and their bedding and excreta were col-

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lected for seven days after inoculation (DAI) and incinerated to kill oocysts that might pass unexcysted in feces (Dubey and Frenkel, 1973). Pheasants had access to poultry ration and water *ad libitum* without any anticoccidial drugs.

Tissues of all pheasants except pheasant number 12, which died 19 DAI, were tested for *T. gondii*. Tissue samples from six pheasants (group B) were pooled for the bioassay. One-half of the brain, most of the heart, and muscles from breast and legs (total 100 g) were pooled and homogenized in five volumes of 0.85% NaCl (saline solution) in a blender. To this an equal volume of 0.52% acid-pepsin solution (Dubey and Beattie, 1988) was added, incubated at 37 C for 90 min in a shaker water bath. The homogenate was filtered, centrifuged at 1,200 × *g*, and washed in saline; the sediment then was suspended in 5 ml antibiotic saline (1,000 units of penicillin and 100 μg of streptomycin/ml saline) as described by Dubey and Beattie (1988). The homogenate was inoculated subcutaneously (SC) into five Swiss Webster outbred female mice weighing 20 to 25 g each. Tissues from the remaining nine pheasants (groups A and C) were tested individually. For this, one-half of the brain, most of the heart, and muscles from breast and legs (100 g) were homogenized in saline. Muscles from breast and legs were digested in acid-pepsin solution, and one-fourth of the digest was tested in five mice for each pheasant. Brains and heart muscles were ground separately in saline solution with a mortar with a pestle, and most of the undigested homogenate was inoculated SC into five mice each.

Mice inoculated with pheasant tissues were bled and killed by cervical dislocation 66 DAI. Serum from each mouse was tested for *T. gondii* antibodies at dilutions of 1:100 using the modified agglutination test (MAT) of Desmonts and Remington (1980). A portion of cerebrum from each mouse was examined microscopically for tissue cysts as described by Dubey and Beattie (1988).

Blood samples from pheasants were collected from the heart or wing vein 1 day before the day of oocyst inoculation, 40 DAI, and at necropsy. Sera were stored at −70 C until the termination of the experiment.

The MAT, latex agglutination test (LAT) and indirect hemagglutination test (IHA) were used to test pheasant sera for *T. gondii* antibodies at the USDA Zoonotic Diseases Laboratory in Beltsville, Maryland (USA). For MAT, sera were initially screened at 1:25, 1:50, and 1:500 dilutions using the formalin-fixed whole tachyzoites as described by Dubey and Desmonts (1987). Sera with antibodies were titrated in two-fold dilutions. The LAT was performed with a commercial test kit following manufacturer's instructions (TOXOTEST-MT, Eiken Chemical Co., San Diego, California, USA). Sera were diluted two-fold 1:16 to 1:4,096; titers <1:32 were considered negative in the LAT. The IHA was performed with a commercial kit following manufacturer's instructions (TPM-TEST, Wampole Laboratories, Cranbury, New Jersey, USA). Sera were diluted two-fold 1:64 to 1:16,394; titers <1:64 were considered negative for the IHA test.

The Sabin-Feldman dye test (Desmonts and Remington, 1980), was performed at the Institut de Puériculture, Paris, France. Sera first were heat-inactivated at 60 C for 30 min and 1:4 to 1:512 dilutions were tested.

Pheasants that died or were killed at the end of the experiment were necropsied. Portions of the brain, both eyes, heart, pectoral (breast) and semitendinosus (leg) muscles, liver, lung, spleen, kidneys, proventriculus, gizzard, and small and large intestines were fixed in 10% formalin. Paraffin-embedded sections were cut at 5 μm thickness, stained with hematoxylin and eosin (H&E), and examined microscopically. Paraffin sections of some tissues were reacted with anti-*T. gondii* serum in an avidin-biotin complex immunohistochemical test using reagents and procedures described by Lindsay and Dubey (1989).

RESULTS

Pheasants inoculated with GT-1 strain of *T. gondii* were anorectic 8 to 14 DAI, were depressed; bird number 12 died 19 DAI. The pheasants inoculated with 10^5 (group A) or 10^4 (group B) oocysts of the ME 49 strain did not manifest clinical signs of infection.

Lesions and *T. gondii* were seen in histological sections of the bird that died 19 DAI. Enteritis, splenic necrosis, myocarditis and encephalitis were the main lesions. There were focal areas of necrosis and mononuclear cell infiltration in skeletal muscles, extensive necrosis in spleen (Fig. 1) and pancreas; *T. gondii* was not seen in histologic sections stained with H&E. A few tachyzoites were seen among necrotic tissue in spleen reacted with anti-*T. gondii* serum. Enteritis was characterized by transmural necrosis and mixed leukocytic cell infiltration from serosa and mucosa. Most of the mucosal epithelium had autolysed and sloughed away from the underlying tissue and numerous bacteria

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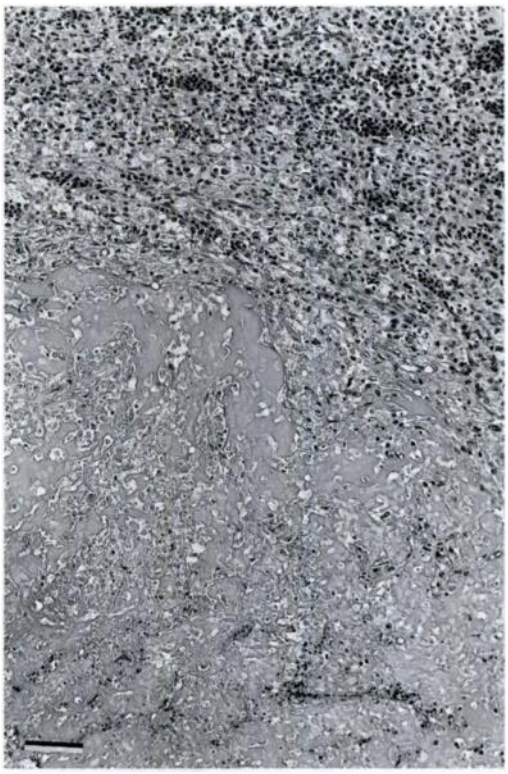

FIGURE 1. Necrosis of parenchyma and blood vessels in spleen of the pheasant that died 19 days after inoculation with *Toxoplasma gondii* oocysts. H&E stain. Bar = 50 μm.

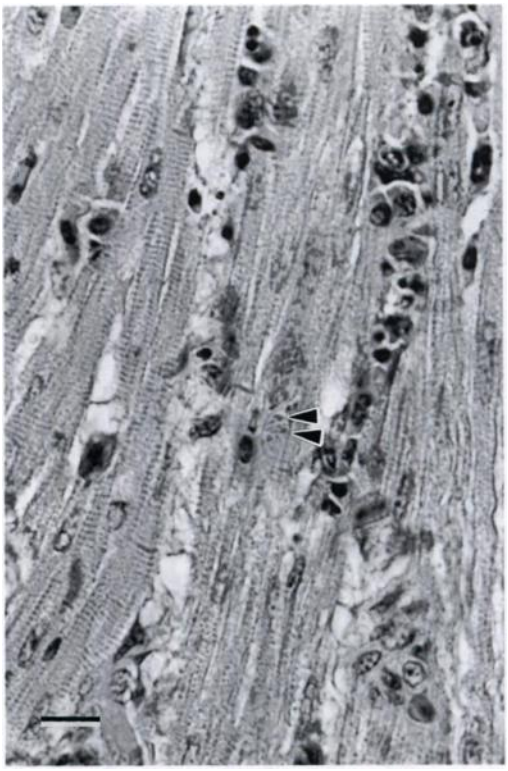

FIGURE 2. *Toxoplasma gondii* tissue cysts containing bradyzoites (arrowheads) and mononuclear cells infiltration in the myocardium of the pheasant that died 19 days after inoculation with *T. gondii* oocysts. H&E stain. Bar = 10 μm.

were in the mucosa. *Toxoplasma gondii* was not seen in sections of intestine.

The myocarditis was characterized by focal necrosis of myocytes, and infiltration of mononuclear cells between individual myocytes, around groups of myocytes and around small blood vessels (Fig. 2). *Toxoplasma gondii* tissue cysts and bradyzoites were seen in lesions. Bacteria also were seen in lesions as well as in small capillaries. Encephalitis was characterized by focal areas of necrosis and hemorrhage, perivascular infiltration of mononuclear cells, and gliosis (Fig. 3). *Toxoplasma gondii* tissue cysts and a few tachyzoites were seen in lesions and away from the lesions (Fig. 4).

Inflammatory lesions without *T. gondii* were seen in brain and muscles of the four remaining pheasants from group C given the GT-1 strain. Encephalitis was characterized by focal accumulation of mononuclear cells around blood vessels and in the neuropil of all pheasants. Myocarditis was characterized by necrosis of myocytes and focal accumulations of lymphocytes. Focal infiltrations of mononuclear cells were seen in ciliary muscles of eyes of two pheasants.

Focal myositis involving ciliary muscles, extraocular muscles, and the myocardium was seen in two of the six pheasants given 10^4 ME 49 oocysts. Lesions and *T. gondii* were not seen in sections of tissue of any of the five pheasants given 10^5 ME 49 oocysts.

Toxoplasma gondii was isolated from the tissues of the nine pheasants killed 68 days after inoculation (Table 1). It was isolated from the brains, hearts, and skeletal muscles of all four pheasants given

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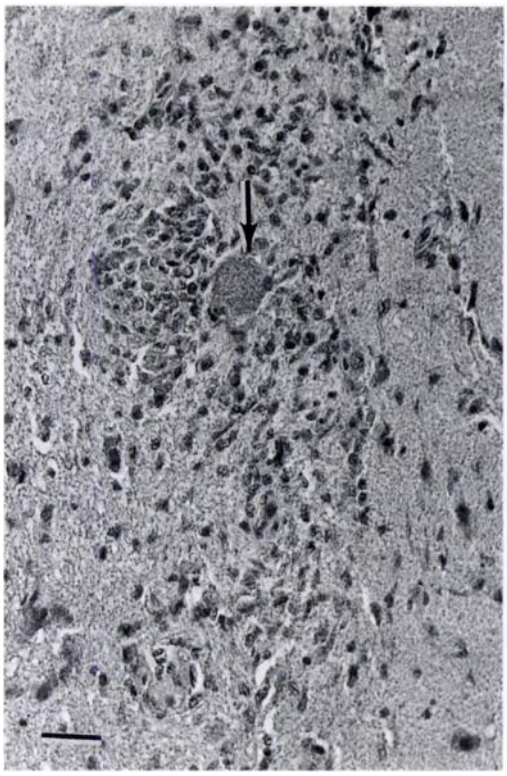

FIGURE 3. *Toxoplasma gondii* tissue cyst (arrow) in a focus of glial cell proliferation of a pheasant. H&E stain. Bar = 22 μm.

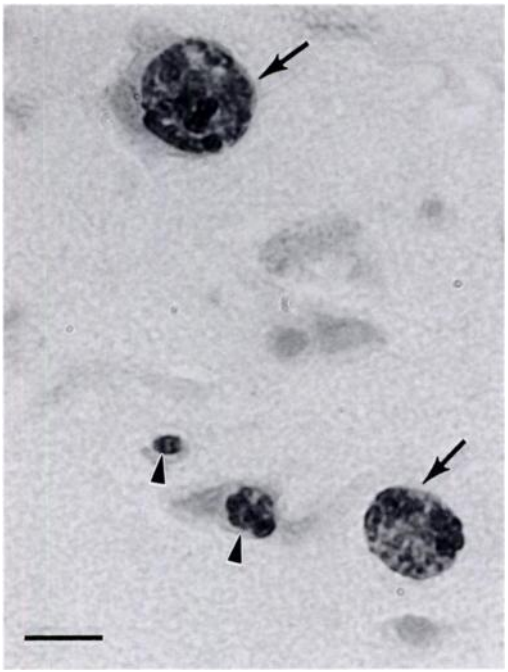

FIGURE 4. *Toxoplasma gondii* tissue cysts (arrows) and tachyzoites (arrowheads) in the cerebrum of the pheasant that died 19 days after *T. gondii* inoculation. Immunohistochemical stain. Bar = 7.5 μm.

GT-1 oocysts. All 60 mice inoculated with pheasant tissues infected with the GT-1 strain died or had to be euthanized by cervical dislocation within 21 DAI; *T. gondii* tachyzoites were found in lung imprints of all 60 mice.

All mice inoculated with tissues of pheasants given the ME 49 strain survived. Twenty-four of the 75 mice inoculated with tissues of pheasants in group A (Table 1) and 15 of 30 mice inoculated with tissues of pheasants from group B (Table 2) were infected with *T. gondii* based on the development of antibody titers of ≥1:100 and the finding of tissue cysts in mice; tissue cysts were found in all seropositive mice. *Toxoplasma gondii* was not recovered from the tissues of pheasant number 6 (Table 2).

All 15 pheasants that survived oocyst inoculation past 35 DAI had developed antibody titers of >1:800 in the MAT by the day of necropsy (Tables 1, 2). Antibody titers in LAT and IHA were lower than the titers in MAT. Antibody titers in MAT, LAT, and IHA in sera of pheasants in groups A and C bled 40 and 68 DAI did not vary more than one serum dilution (data not shown). Dye test antibodies were not detected at a 1:4 dilution of the sera in any of the 15 pheasants. The antibody titers with MAT of the positive control sera provided in LAT and IHA kits were 1:25,600 and 1:3,200, respectively, as compared to stated titers of 1:256 and 1:512 in the respective kits.

DISCUSSION

From our results pheasants appear to be relatively resistant hosts to clinical toxoplasmosis. The pheasants given 10^4 oocysts of the GT-1 strain developed few clinical signs and only one died. The GT-1 strain of *T. gondii* is highly virulent in mice and

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TABLE 1. Serological responses and presence of *T. gondii* in pheasants given oocysts of the GT-1 or ME 49 strain.

Pheasant number	Isolation of *T. gondii* in mice[a]			Reciprocal antibody titers[b] with different serological tests		
	Brain	Heart	Skeletal muscle	MAT	IHA	LAT
1[c]	2	0	1	3,200	64	128
2[c]	1	0	2	3,200	64	128
3[c]	0	2	2	3,200	128	256
4[c]	2	1	0	800	<64	32
5[c]	5	4	2	3,200	128	128
13[d]	5	5	5	25,600	2,048	1,024
14[d]	5	5	5	6,400	128	256
15[d]	5	5	5	25,600	8,192	2,048
16[d]	5	5	5	6,400	256	512

[a] Number of mice positive for *T. gondii* of five mice inoculated with pheasant tissues.

[b] MAT, modified agglutination test; IHA, indirect hemagglutination test; LAT, latex agglutination test. Sera were obtained 68 days after inoculation.

[c] Given 10^5 oocysts of the ME 49 strain.

[d] Given 10^4 oocysts of the GT-1 strain.

TABLE 2. Serological responses and presence of *Toxoplasma gondii* in pheasants given 10^4 oocysts of the ME 49 strain.

Pheasant number	Isolation of *T. gondii* in mice[a]	Reciprocal antibody titers with different serological tests[b]		
		MAT	IHA	LAT
6	0	6,400	128	128
7	2	1,600	64	128
8	5	3,200	512	256
9	2	1,600	<64	128
10	2	3,200	<64	128
11	4	3,200	<64	128

[a] Number of mice positive for *T. gondii* of five mice inoculated with pooled pheasant tissues obtained on day 36 after inoculation.

[b] MAT, modified agglutination test; IHA, indirect hemagglutination test; LAT, latex agglutination test. Sera were obtained 36 days after inoculation.

can be lethal in adult sheep, goats, and antelopes (Dubey and Beattie, 1988). No pheasant inoculated with ME 49 strain of *T. gondii* developed clinical signs, although *T. gondii* persisted in tissues of 10 of 11 pheasants.

Toxoplasma gondii within lesions were demonstrable only in the pheasant that died 19 DAI. Some of the degenerating *T. gondii* in the heart were bradyzoites and no tachyzoites were seen. Bradyzoites could be recognized in histologic sections because of their terminal nucleus; the nucleus in tachyzoites is located centrally (Dubey and Beattie, 1988). Severe necrosis in the intestines of the pheasant that died 19 DAI probably was caused by *T. gondii*, as tachyzoites, apparently killed by host response, were present in these organs. The inflammatory lesions in the heart and brain of some of the pheasants killed 68 DAI probably were due to *T. gondii*, although *T. gondii* was not seen in histological sections. The finding of muscular lesions in two of the six birds given 10^4 ME 49 oocysts and killed 36 DAI and not in the five birds given 10^5 ME 49 oocysts and killed 68 DAI may have been related to the day of necropsy after *T. gondii* inoculation.

Based on our results, the MAT was more suitable than other tests for detecting antibodies to *T. gondii* in sera of pheasants. Using the MAT, we detected antibodies sooner and in higher titers than with the IHA and LAT. The dye test was unsuitable for detecting *T. gondii* antibodies which is in agreement with results obtained with chicken sera (Frenkel, 1981).

Based on our results, *T. gondii* can persist in edible tissues of pheasants. Therefore, pheasant meat should be cooked thoroughly before human or animal consumption. Because *T. gondii* is disseminated in the environment through feces of infected Felidae, cats should not have access to food and water consumed by pheasants.

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