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Authors: HASLETT, THERESA M., and SCHNEIDER, WARREN J.

Source: Journal of Wildlife Diseases, 14(2) : 173-175

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-14.2.173>

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OCCURRENCE AND ATTEMPTED TRANSMISSION OF *Toxoplasma gondii* IN EUROPEAN STARLINGS (*Sturnus vulgaris*).

THERESA M. HASLETT and WARREN J. SCHNEIDER, Department of Biological Sciences and the School of Pharmacy, University of the Pacific, Stockton, California 95211, USA.

Abstract: Serums of 563 fledgling starlings (*Sturnus vulgaris*) trapped during a 14 month period near Lodi, California were examined for antibodies to *Toxoplasma gondii* using the microtiter indirect hemagglutination method. Titers ranging from 1:64 to 1:512 were found in 4.8% of the birds. Starlings collected during May through October had a higher prevalence of antibody than those collected during November through April. Rats inoculated with individual heart and brain suspensions from 10 seropositive starlings remained seronegative for *T. gondii* antibodies when tested at 22 and 82 days post-inoculation. Peritoneal smears made from these rats at post-inoculation day 82 were negative for *T. gondii*.

INTRODUCTION

Toxoplasmosis, caused by the protozoan *Toxoplasma gondii*, affects humans and a wide variety of lower animals. This disease is medically important because it can cause congenital defects in infants born to infected mothers.² Human infections are believed to originate primarily from two sources; (1) ingestion of infective oocysts from cat feces and (2) ingestion of viable cysts contained in raw or undercooked meat.¹

In a recent survey of food-producing animals in California, 24 to 32% of 353 animals tested were seropositive for *T. gondii*.³ Investigators noted a dearth of domestic cats, considered to be the primary amplifying host of toxoplasmosis, in several areas from which seropositive animals originated. They suggested wildlife as a potential reservoir of *T. gondii*.

Vast numbers of European starlings (*Sturnus vulgaris*) now populate the United States. The possible involvement of starlings in disease transmission is discussed elsewhere.⁴ The experiment reported here is an initial attempt to

determine if starlings may be infected with *T. gondii*, thus acting as a wildlife reservoir of the disease and forming a link in the chain which leads to human infection.

MATERIALS AND METHODS

A total of 563 fledgling starlings were trapped during the period September, 1975 through October, 1976.[□] The 141 birds trapped in September and October, 1975 were tested for serum antibodies against *T. gondii* but transmission experiments were not performed. Beginning in November 1975, all birds trapped were tested for serum antibodies, and tissues from all seropositive starlings (10 in number) were used in transmission experiments.

Starlings were collected from stationary traps placed in vineyards and a dairy operation on nine ranches located within a 6.5 km radius near Lodi, California. The area includes pasture as well as major crops such as grapes, corn, tomatoes and bell peppers. Livestock includes dairy and beef cattle, sheep and

[□] Starlings were trapped and supplied by Mr. Marvin Switzenberg, Agricultural Commissioner in charge of the San Joaquin County, California, starling control project.

horses. The starling collection area is unrelated to the California survey of food producing animals mentioned in the introduction.

Blood samples were collected via cardiac puncture. After clotting, the blood was centrifuged and the serum was tested for antibodies against *T. gondii* using the microtiter indirect hemagglutination inhibition (IHA) test utilizing commercial antigen.² Non-specific antibodies were removed by using an absorbent provided in the commercial test kit. Starlings with end titers of $\geq 1:64$ were considered positive.

Beginning in November 1975, carcasses were retained at 40 C after collection of blood samples. Within 24 h., 1 g of cardiac and brain tissue from each seropositive starling was used to make an individual 20% suspension in 0.9% sterile saline. One ml of the suspension from each individual seropositive starling was inoculated intraperitoneally into 2 Sprague-Dawley rats and a third rat was inoculated with sterile saline as a control. All rats were examined prior to inoculation and shown to be free of

antibodies against *T. gondii*. Twenty-two and 82 days after inoculation, blood samples were again taken from the rats and tested for antibodies to *T. gondii*. Eighty-two days after inoculation the rats were examined at necropsy and peritoneal smears were stained with Giemsa to facilitate finding *Toxoplasma*.

RESULTS

Of 563 starlings collected, 27 (4.8%) were seropositive for *T. gondii*. Nineteen had end titers of 1:64, 2 had end titers of 1:128, 4 had end titers of 1:256, and 2 had end titers of 1:512.

The number of starlings collected and the number of seropositive starlings were recorded with respect to location and time of collection so that prevalence could be calculated for each variable. Table 1 shows the prevalence based upon time of trapping.

Seropositive starlings were found among the birds from 7 of the 9 trapping locations. Among the locations which yielded seropositive starlings, the prevalence ranged from 2% to 17%.

TABLE 1. Prevalence of antibodies against *Toxoplasma gondii* in starlings.

Month	Number of Starlings Tested	Number of Seropositive Starlings	% Prevalence of Antibodies
September 1975	81	12	15
October 1975	60	5	8
November 1975	39	0	0
December 1975	25	0	0
January 1976	67	2	3
February 1976	29	0	0
March 1976	36	0	0
April 1976	24	0	0
May 1976	30	4	13
June 1976	41	1	2
July 1976	39	0	0
August 1976	21	0	0
September 1976	38	2	5
October 1976	33	1	3

² Industrial Biological Laboratories, Inc., Rockville, Maryland.

The 10 control rats and the 20 rats inoculated with tissues from seropositive starlings were seronegative 22 and 82 days after inoculation. Peritoneal smears made from these rats at postinoculation day 82 were negative for *T. gondii*.

DISCUSSION

Prevalence of seropositive starlings was not uniformly distributed throughout the 14 month collection period. Prevalence was higher during the May-October period (characterized by higher temperatures and little or no rainfall) and lower during the November-April period (lower temperatures and greater rainfall).

Conclusions regarding the location of seropositive starlings could not be made because of two factors: (1) birds were not

collected at a uniform rate from each ranch during the test period; and (2) birds probably ranged throughout the collection area during the test period.

Since the rats were seronegative and did not develop signs of *toxoplasmosis*: (1) the positive reactions of the starlings to the IHA test were "false-positives", i.e., caused by a cross-reacting organism or non-specific substance not eliminated during the absorption phase of the IHA test; (2) the starlings were infected, but the heart and brain tissues used did not contain infective *T. gondii*; or (3) strains of *T. gondii* may exist that are specific for starlings and unable to survive in rats.

The results of this experiment indicate that a small percentage of starlings have serum antibodies to the *toxoplasmosis* IHA test. Further investigations are needed to clarify the role, if any, that starlings play in the perpetuation and transmission of *toxoplasmosis*.

Acknowledgements

The authors thank Dr. Fuad Nahhas for guidance and Mr. Darryl Behymer for technical assistance.

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Received for publication 28 March 1977