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Author: Burgess, Elizabeth C.

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EXPERIMENTAL INOCULATION OF MALLARD DUCKS (*ANAS PLATYRHYNCHOS PLATYRHYNCHOS*) WITH *BORRELIA BURGDORFERI*

Elizabeth C. Burgess
University of Wisconsin, School of Veterinary Medicine, 2015 Linden Drive West, Madison, Wisconsin 53706, USA

ABSTRACT: Birds have been incriminated as disseminaters of *Borrelia burgdorferi* and have the potential to spread the organism over a wide geographic range. *Borrelia burgdorferi* has been isolated from the liver and blood of passerine birds and from *Ixodes dammini* removed from passerines. The objective of this study was to determine if waterfowl, specifically mallards (*Anas platyrhynchos platyrhynchos*), were susceptible to infection with *B. burgdorferi*. Eight ducks were inoculated with *B. burgdorferi*; four orally and four intravenously (i.v.) and two ducks were inoculated with phosphate buffered saline as controls. All eight inoculated birds became infected and developed antibodies to *B. burgdorferi*. The spirochete was isolated from cloacal material from an orally infected duck on day 22 postinoculation (PI) and from an i.v. infected bird on day 29 PI, from the blood of an i.v. infected bird on day 7 PI, and from the kidney of an orally infected bird. *Borrelia burgdorferi* was detected by indirect immunofluorescence using the *B. burgdorferi* specific monoclonal antibody H5332 in kidneys of three orally infected birds and one i.v. infected bird and from the mesentery of one orally infected bird. These findings show that mallard ducks are susceptible to infection by *B. burgdorferi* and that they can be infected orally and shed the organism in the droppings. Thus, mallards could disseminate *B. burgdorferi* over long distances without the need of an arthropod vector.

Key words: *Borrelia burgdorferi*, mallard, *Anas platyrhynchos platyrhynchos*, Lyme disease, oral infection, experimental infection

INTRODUCTION

Lyme disease was first recognized in the United States at Lyme, Connecticut in 1975 (Steere et al., 1977). The causative spirochete (*Borrelia burgdorferi*) is transmitted primarily by the bite of an ixodid tick, *Ixodes dammini* (Burgdorfer et al., 1982). The disease was originally limited to the eastern coastal, midwestern, and far western states. Since 1975 the geographic distribution of the disease has increased to 32 states (Ciesielski, 1988). Birds have been incriminated in the spread of *B. burgdorferi* infected ticks. Immature *I. dammini* are known to parasitize at least 24 species of passerine birds and *B. burgdorferi* has been isolated from the blood of passerine birds and from ticks removed from them (Anderson and Magnarelli, 1984; Anderson et al., 1986; Battely et al., 1987). There have been studies on the susceptibility of waterfowl to *B. burgdorferi*. Waterfowl migrate long distances and have the potential to transmit the spirochete over wide geographic areas. The objectives of this study were: (1) to determine if mallards (*Anas platyrhynchos platyrhynchos*) could be infected with *B. burgdorferi* and, if so, by what routes; and (2) if the spirochete could be isolated from the blood or droppings of infected mallards and, if so, for what duration.

MATERIALS AND METHODS

Ten adult male mallard ducks obtained from a game farm (Tall Oaks, Park Rapids, Minnesota 56470, USA) were housed in individual stainless steel cages in an arthropod-free room. Eight birds were inoculated with a culture of *B. burgdorferi* originally isolated from the blood of a *Peromyscus maniculatus* (Burgess et al., 1986) and passaged eight times in culture medium. Four of the eight mallards were inoculated orally by dropping 0.5 ml of BSK II medium (Barbour, 1984) containing approximately 4,000 spirochetes (determined by serial dilution in a counting chamber) into the back of the mouth and the other four were inoculated intravenously (i.v.) with 1,000 spirochetes in 0.2 ml of phosphate buffered saline (PBS). Two ducks were used as controls, one inoculated orally with 0.5

TABLE 1. *Borrelia burgdorferi* inoculation route, dose, indirect immunofluorescent antibody (IFA) titers at day 28 post inoculation, *B. burgdorferi* culture results and results of tissues stained (IF) with monoclonal antibody H5332 and fluorescein conjugated rabbit anti-mouse IgG in 10 Mallard ducks (*Anas platyrhynchos platyrhynchos*).

Duck number	Route	Dose	IFA titer	Culture	IFA staining
1	i.v.	0.2 ml PBS[a]	1:8	Negative	Negative
2	Oral	0.5 ml PBS	1:8	Negative	Negative
3	Oral	4,000[b] spirochetes	1:256	Negative	Kidney Mesentery
4	Oral	4,000 spirochetes	1:256	Cloaca[c]	Kidney
5	Oral	4,000 spirochetes	1:256	Kidney	Kidney
6	Oral	4,000 spirochetes	1:256	Negative	Negative
7	i.v.	1,000[d] spirochetes	1:256	Blood[e]	Negative
8	i.v.	1,000 spirochetes	1:256	Cloaca[f]	Negative
9	i.v.	1,000 spirochetes	1:512	Negative	Negative
10	i.v.	1,000 spirochetes	1:512	Negative	Kidney

[a] Phosphate buffered saline.
[b] Spirochetes in 0.5 ml PBS.
[c] Day 22 postinoculation.
[d] Spirochetes in 0.1 ml PBS.
[e] Day 7 postinoculation.
[f] Day 29 postinoculation.

ml of BSK media and one i.v. with 0.5 ml of PBS (Table 1).

Blood and cloacal samples were collected for culture attempts on days 2, 4, 7, 14, 22, 29 and 43 postinoculation (PI) and sera from day 28 PI was saved for antibody testing. Cloacal samples for culture attempts were taken by inserting a cotton tipped swab into the cloaca and gently turning. Isolation attempts for *B. burgdorferi* were performed by inoculating 0.1 ml of blood or by placing the swab taken from the cloaca into culture tubes containing 7 ml of BSK II medium. Samples were examined weekly by placing one drop from each tube onto a microscope slide and observing by darkfield microscopy for the presence of spirochetes. Spirochetes were identified by immunofluorescence using the monoclonal antibody H5332 (obtained from E. Bosler, New York State Department of Health, Health Sciences Center, Stony Brook, New York 11794, USA) and then with fluorescein labelled goat anti-mouse IgG (Cappell Worthinton Laboratories, Malvern, Pennsylvania 19355, USA). All of the ducks were killed and necropsied on day 43 PI. The kidney, spleen, liver and heart of each duck was saved and one-half of each organ was triturated with 2 ml of BSK medium in a Ten-broeck tissue grinder (Bellco Glass, Vineland, New Jersey 08360, USA) and 1 ml inoculated into culture tubes. The remaining tissue was fixed in 10% buffered formalin; paraffin embedded 7 μm sections were cut and these were slide mounted and deparaffinized. Sections were then stained with mouse monoclonal antibody to *B. burgdorferi* H5332 to identify any spirochetes. Antibody titers to *B. burgdorferi* and *Borrelia anserina* (a closely related spirochete) were determined by means of an indirect immunofluorescent antibody test (IFA), using previously described methods (Burgess et al., 1986). For *B. burgdorferi* testing the eighth passage *P. maniculatus* isolate was used as the antigen and for *B. anserina* testing the spirochete used was obtained from R. Johnson (Department of Microbiology, University of Minnesota, Minneapolis, Minnesota 55455, USA). The birds were tested also for *B. anserina* antibodies prior to infection with *B. burgdorferi*.

Fluorescein isothiocyanate (FITC) conjugated rabbit anti-duck whole serum was prepared by inoculation of rabbits with whole mallard duck serum using a procedure previously described (Kawamura, 1977). This conjugated sera was then used in the IFA tests to determine *B. burgdorferi* antibody levels in the duck sera.

RESULTS

Ten of 10 ducks had IFA titers of <1:64 for both *B. burgdorferi* and *B. anserina* and the blood and cloacal cultures were negative for both species prior to inoculation. The eight ducks inoculated with *B. burgdorferi* developed antibodies to *B. burgdorferi* at titers from 1:128 to 1:512 by day 29 PI while the titers in the control ducks remained at <1:64 (Table 1). The

results in the infected birds and control birds are presented in Table 1. *Borrelia burgdorferi* was cultured from the cloaca of an orally infected bird on day 22 PI and from an i.v. infected bird on day 29 PI, from the blood of an IV infected bird on day seven PI and from the kidney of an orally infected bird. *Borrelia burgdorferi* was detected by IFA using the monoclonal antibody in the kidney of three orally infected birds and one i.v. infected bird and from the mesentery of one orally infected bird. No antibodies to either *B. burgdorferi* or *B. anserina* were detected in the two control ducks. Spirochetes were not cultured from the control ducks.

DISCUSSION

My results show that mallards (1) are susceptible to infection with *B. burgdorferi* by both the oral and i.v. routes, (2) can become persistently infected for at least 43 days, (3) develop a spirochetemia, and (4) can shed spirochetes in the droppings for at least 29 days. Mallards were chosen for this study because they are the most abundant and most widely distributed duck in the neoartic; thus, they are an ideal candidate for disseminating spirochetes over a wide geographic area (Bellrose, 1976). The major migration route used by two million mallards extends from southeastern Saskatchewan to northwestern Illinois and then south to Tennessee, eastern Arkansas, and Mississippi (Bellrose, 1976). Birds migrating on this flyway go through endemic areas for Lyme disease in Minnesota and Wisconsin where they stop and feed during their spring and fall migration. *Ixodes dammini* has been reported by duck hunters to be frequently encountered on grass around pot holes; these ticks have been collected from grass bordering ponds and pot holes in Wisconsin (Jackson and DeFoliart, 1970). If mallards become infected with *B. burgdorferi* during the time spent in these areas they could subsequently disseminate the spirochete during migration. Because spirochetes were shed as long as 29 days in the present study, it is possible that migrating birds could spread spirochetes the entire length of this migration route. During migration, birds travel in large groups and are often crowded together. At the Horicon Marsh in Wisconsin, (43°5′W, 88°5′N) there may be as many as 100,000 ducks during a single time period. Such crowding facilitates transmission of fecal–oral transmitted diseases (Burgess and Yuill, 1983).

Another *Borrelia* sp. infection of birds, *B. anserina*, was first recognized in geese (Hofstad et al., 1984) and is transmitted by the bite of *Argas persicus* or by ingestion of infected ticks. This spirochete can be transmitted directly from bird to bird by ingestion of infected droppings (Hofstad et al., 1984). The findings in the present study show that *B. burgdorferi* also can be orally transmitted.

If free-flying ducks or other birds can become infected with *B. burgdorferi* by the oral route and can shed the spirochete in the droppings, this could be an important means of spread over long distances without the need of a tick vector to maintain the infection. Ducks in this study did not develop any clinical signs such as lameness or swollen joints. It remains to be determined if *B. burgdorferi* infection in wild birds can cause disease. Further research is needed to determine the extent of infection in free-flying waterfowl and if infections can result in clinical illness in these birds.

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