

## **EXPERIMENTAL INFECTION OF NORTHERN BOBWHITE QUAIL WITH BORRELIA BURGdorFERI**

Authors: Bishop, Kathrine L., Khan, Mazhar I., and Nielsen, Svend W.

Source: Journal of Wildlife Diseases, 30(4) : 506-513

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-30.4.506>

---

BioOne Complete ([complete.BioOne.org](https://complete.BioOne.org)) is a full-text database of 200 subscribed and open-access titles in the biological, ecological, and environmental sciences published by nonprofit societies, associations, museums, institutions, and presses.

Your use of this PDF, the BioOne Complete website, and all posted and associated content indicates your acceptance of BioOne's Terms of Use, available at [www.bioone.org/terms-of-use](https://www.bioone.org/terms-of-use).

Usage of BioOne Complete content is strictly limited to personal, educational, and non - commercial use. Commercial inquiries or rights and permissions requests should be directed to the individual publisher as copyright holder.

---

BioOne sees sustainable scholarly publishing as an inherently collaborative enterprise connecting authors, nonprofit publishers, academic institutions, research libraries, and research funders in the common goal of maximizing access to critical research.

## EXPERIMENTAL INFECTION OF NORTHERN BOBWHITE QUAIL WITH *BORRELIA BURGENDORFERI*

Kathrine L. Bishop,<sup>1</sup> Mazhar I. Khan,<sup>2</sup> and Svend W. Nielsen<sup>1</sup>

<sup>1</sup> Center for Wildlife Diseases, Department of Pathobiology, University of Connecticut, Storrs, Connecticut 06268, USA

<sup>2</sup> Department of Pathobiology, University of Connecticut, Storrs, Connecticut 06268, USA

**ABSTRACT:** Four-week-old northern bobwhite quail (*Colinus virginianus*) were inoculated subcutaneously with 10<sup>6</sup> organisms from a low passage culture of *Borrelia burgdorferi*. Blood was collected weekly for culture, antibody detection, and immunoblot analysis. Three weeks post-inoculation, viable spirochetes were isolated from the blood of one bird, but not from kidney, spleen, liver, or heart; all infected birds from which preinfection antibody titer had been established, had antibodies by the enzyme-linked immunosorbent assay (ELISA). The inoculated birds did not show clinical signs of disease and there were no detectable gross or histopathologic lesions. *Borrelia burgdorferi* was detected in sections of kidneys on fluorescent antibody tests. Using a polymerase chain reaction (PCR) analysis to detect *Borrelia burgdorferi* DNA in tissue samples, the expected PCR product (DNA) of 246 base pairs was visible on agarose gel stained with ethidium bromide. The identity of the PCR product was confirmed by slot blot hybridization with *Borrelia burgdorferi* specific DNA probe. Thus, these birds sustained infections for at least 3 weeks without clinical signs and may play a role in the transmission of *Borrelia burgdorferi*.

**Key words:** *Borrelia burgdorferi*, northern bobwhite quail, *Colinus virginianus*, serology, pathology, polymerase chain reaction.

### INTRODUCTION

Lyme borreliosis, a disease affecting both man and animals, is caused by the spirochete *Borrelia burgdorferi* and is transmitted by several species of Ixodid ticks (Burgdorfer, 1984). In the northeastern United States where the disease is enzootic, the primary vector is *Ixodes scapularis* (formerly *I. dammini*) which feeds on many mammals, most importantly the white-footed mouse (*Peromyscus leucopus*) and the white-tailed deer (*Odocoileus virginianus*). The tick also has been associated with many species of ground feeding birds, but the role of birds in the transmission of the disease is not clearly understood. Their feeding and migratory habits of birds present them as potential hosts, both as passive carriers of the tick and as reservoirs of the spirochete. Errant birds from northern Europe (Shwartz, 1987), where the disease has been recognized since the turn of the century, may have been a means of introduction of the disease by infected ticks or spirochetes to the northeastern United States. Avian transmission also may explain the three geographically separate areas of endemic

Lyme borreliosis determined within a few years of its initial discovery in this country. These regions include the shores of Long Island sound, Cape Cod, Massachusetts, and the state of Wisconsin (USA) (McLean et al., 1993).

Our objective was to determine the pathogenicity of *B. burgdorferi* in birds. We experimentally inoculated northern bobwhite quail (*Colinus virginianus*) with *B. burgdorferi* to establish infectivity, immune response and pathological changes, and to attempt to detect spirochetes in blood and tissues by bacterial culture, the fluorescent antibody (FA) test, and polymerase chain reaction (PCR) techniques.

### MATERIALS AND METHODS

The northern bobwhite quail was selected as the experimental subject for this study because it is a woodland, ground dwelling species and is commercially available. Thirty 4-wk-old northern bobwhite quail (Ronald Urban, Eastford, Connecticut, USA) were allowed to acclimate for a period of 2 wk before starting the experiment. Each bird was individually identified. The experimental infection study started in early October 1990 and ended in December 1991.

The *B. burgdorferi* culture used to infect 24

birds was derived from a tick foregut isolate of third passage #25550-3, which was provided by Dr. John Anderson, Department of Entomology, Connecticut Agricultural Station, New Haven, Connecticut. The cultures were grown and maintained in Barbour-Stoenner-Kelly (BSK) media (Barbour, 1984). Pre-infection blood samples were collected from the wing veins of the birds. Twenty-four birds were inoculated subcutaneously with approximately  $10^6$  organisms of the above low-passage *B. burgdorferi* suspended in 7 ml of BSK media. Blood was collected weekly and approximately 0.25 ml was cultured in 7 ml of BSK media containing 20 mg/ml rifampicin (Sigma Chemical Company, St. Louis, Missouri, USA). The tubes were incubated at 34 C and wetmounts of the cultures were checked weekly for 3 mo under darkfield microscopy (Model BH-2, Olympus Optical Company, Tokyo, Japan). Six birds were inoculated subcutaneously with 0.2 ml of BSK media for use as negative controls.

The serum samples from 29 birds, 24 infected and 5 controls, were tested in triplicate by enzyme-linked immunosorbent assay (ELISA) as described by Magnarelli et al. (1984). The *B. burgdorferi* antigen was derived from the culture (25550-3) used to infect the birds and was prepared by washing the organisms three times with 0.1% merthiolate in phosphate buffer saline (PBS), pH 7.2, centrifuged at  $10,000 \times G$  for 30 min each time. The organisms were resuspended in PBS, and standardized spectrophotometrically to an absorbance of 0.025 at 650 nm ( $A_{650} = 0.025$ ). Microtiter plates (Dynatech Labs, Alexandria, Virginia, USA) were coated with 50  $\mu$ l/well ( $A_{650} = 0.01$ ) of antigen or 50  $\mu$ l of the PBS with merthiolate (1:10,000) and incubated for 20 hr at 4 C. The plates were then used for the ELISA or stored at -70 C for up to 1 mo. We added 200  $\mu$ l/well of 0.5% horse serum as a blocking solution, incubated the plates for 1 hr at 37 C, and washed them three times in PBS containing 0.05% Tween 20 (Sigma Chemical Company) solution. A two fold serial dilution of bird serum starting at 1:40 was made in PBS containing 0.05% Tween 20, 5% horse serum, and a 1% solution of dextran sulfate (Sigma Chemical Company). Sixty microliters of each dilution was added in triplicate, containing either antigen or PBS (control), followed by a 45 min incubation at 25 C and washed. Horseradish peroxidase conjugated with goat anti-chicken IgG containing both the heavy chain and light chain (1:400) (Kirkegaard and Perry Laboratory Inc. Gaithersburg, Maryland, USA) was added to each well. Incubation was carried out for 45 min at 37 C and washed three times with PBS. We added 60  $\mu$ l/well of ABTS [2,2'-azino-di-(3-ethylbenzthiazoline sulfonate)] per-

oxidase substrate (Kirkegaard and Perry Lab) to the wells, incubated them for 50 min at 37 C, and read the plates on a Microplate Reader (Dynatech Lab) at 410 nm. The absorbance of the control wells was subtracted from the absorbance of the corresponding antigen coated wells to obtain a net value.

We compared the third passage of *B. burgdorferi* BB(25550-3) with the BB reisolated from the blood of infected birds 3 wk post-inoculation with an immunoblot analysis. The organisms were grown in 25 ml BSK medium for 36 to 48 hrs at 37 C. The cells were centrifuged at  $15,000 \times G$  for 25 min and washed four times in PBS. Protein concentration was estimated by the Bradford method (Bio-Rad, Richmond, California, USA). One-hundred micrograms of borrelia cell protein was mixed in 100  $\mu$ l of sample buffer (2% sodium dodecyl sulfate (SDS), 5% 2-mercaptoethanol, 10% glycerol, 62.5 mM Tris pH 6.8, 0.001% bromphenol blue) (Sigma Chemical Company), which was boiled for 5 min along with a molecular weight marker (Bio-Rad); samples were subjected to electrophoresis using a 10% sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE) (Laemmli, 1970). Electrophoresis was done at 50 mA at 4 C until the bromophenol blue dye reached 1 cm from the bottom of the gel. The gels had been pre-equilibrated in transfer buffer (48 mM Tris-Base) (Sigma Chemical Company), 39 mM glycine (Sigma Chemical Company), 20% (v/v) methanol pH 8.3 and 0.037% SDS for 30 min, and transferred onto nitrocellulose membrane (Bio-Rad) at 70 volts for 2.5 hr using a Trans-Blot Electrophoretic Transfer Cell (Bio-Rad) as described by Towbin et al. (1979). The membranes were stained with Ponceau S (Sigma Chemical Company) to view the banding patterns. The membranes were cut in strips for immunoblotting, blocked with 2% bovine serum albumin and 1% calf serum in saline, shaken for 1 hr at 25 C and washed three times (10 min each) in washing buffer (0.4% Tween 20 in PBS). Antisera (1:100 dilution) from three infected birds and from non-infected birds were incubated for 1 hr at 25 C. Alkaline phosphatase labeled- affinity purified goat anti-chicken immunoglobulin G (IgG) (H+L) (Kirkegaard and Perry Lab Inc.) diluted 1:2500 with blotting buffer was incubated for 2 hr at 25 C by rotating gently. The strips were washed briefly in PBS and then allowed to react with BCIP/NBT phosphatase substrate (Kirkegaard and Perry Lab) until bands appeared. The reaction was stopped by washing with distilled water. The strips were carefully blotted with filter paper and stored in a dry dark place at 25 C.

Three weeks post-infection and weekly thereafter four infected birds and one control bird

were killed by cervical dislocation. Prior to their death a blood sample was obtained from a heart puncture. One-half cc of blood was added to 6ml of BSK media and incubated at 34C; the remainder was used to harvest the serum for determination of antibody response. The birds were checked for gross lesions; pieces of kidney, liver, heart, skin, and spleen were aseptically removed and transferred into 7ml of sterile BSK media containing 20 mg/ml rifampicin. Samples of each tissue were also fixed in 10% neutral buffered formalin (Surgipath, Grace Lake, Illinois, USA). Freezing samples for fluorescent antibody assay, histopathology, and PCR was accomplished by adding tissues samples from each bird to a 5ml Nalgene cup (Nalgene Laboratories, Rochester, New York, USA) containing omnifix (An-Con Genetics Inc. Melville, New York). They were covered and stored at -20 C.

Tissue sections from kidney, liver, heart, skin, and spleen were fixed in 10% formalin for 24 hr and embedded in paraffin, and 5  $\mu$ m sections were stained with Steiners Silver (Swisher, 1987) and hematoxylin and eosin (H&E) (Sigma Chemical Co.) (Hrapchak, 1980). Five-micrometer sections of frozen tissue sections (kidney, liver, heart, skin and spleen) from non-infected and infected birds were transferred to poly-L-lysine slides (Sigma Chemical Company) and stored at -70 C. A positive control was created by injecting chicken liver with  $10^5$  live *B. burgdorferi* derived from the same culture which had been used to infect the birds. A direct fluorescent antibody (FA) test was done on frozen tissue sections as described by Hrapchak (1980). Each tissue section was placed on a slide and was dipped into cold acetone and allowed to dry. Two drops of fluorescein isothiocyanate (FITC) conjugated polyclonal antibody (goat-anti-borrelia) (1:40 dilution) (Kirkegaard and Perry Lab) were placed on to the tissue sample and incubated in a moisture chamber for 30 min at 37 C. Each slide was washed with PBS and examined under the fluorescent microscope (Olympus model BH-2).

The DNA was isolated from the originally described third passage culture of *B. burgdorferi* by the procedure of Sambrook et al. (1989). The borrelia cultures were grown in BSK media and centrifuged in 50 ml Nalgene (Fisher Scientific, Medford, Massachusetts) tubes at  $7,000 \times G$  for 15 min. The pellet was lysed with 3 ml of a buffer containing 10 mM Tris and 1 mM ethylene-diaminetetra-acetic acid (EDTA) (TE buffer) with SDS added to a 1% final concentration. Ribonuclease A (Bethesda Research Laboratory (BRL), Bethesda, Maryland) was added to a final concentration of 25  $\mu$ g/ml and incubated for 20 min at 25 C, followed by the addition of 25  $\mu$ g/ml proteinase K (Sigma

Chemical Company) and an incubation of 1 hr at 37 C. The DNA was extracted once with an equal volume of phenol and once with an equal volume of a 1:24 mixture of chloroform and isopropanol. Centrifugation was at  $7,000 \times G$  for 5 min. The supernatant was placed in corex tubes (Fisher Scientific) and the DNA was precipitated with 1/10 volume of 3 M sodium acetate. Two volumes of 100% ethanol were added and incubated overnight at -20 C. The DNA extract then was centrifuged at  $7,000 \times G$  for 10 min and the pellet was resuspended in 300  $\mu$ l of TE buffer, pH 7.6. The DNA concentration was estimated spectrophotometrically (Sambrook et al., 1989) and stored at -20 C.

Deoxyribonucleic acid was isolated from tissues of infected and non-infected birds, and from chicken liver injected with *B. burgdorferi* (Rogers et al., 1990). Fifteen milligrams of tissue were mixed in 1 ml of a buffer containing 0.5 M Tris pH 8.0, 0.02 M EDTA, and 0.01 M NaCl (TEN buffer) and incubated for 3 hr at 55 C. Sodium dodecyl sulfate then was added to a final concentration of 1% to each tube and mixed thoroughly. Proteinase K was added to a final concentration of 25  $\mu$ g/ml and incubated overnight at 50 C. Starting with the phenol extraction, the remainder of the procedure was as before.

The *B. burgdorferi* primer pairs used in PCR were described by Malloy et al. (1990) and were synthesized by Biotechnical Services of the University of Connecticut, Storrs, Connecticut, as described by Beaucage and Caruthers (1981). The PCR was carried out using reagents from GeneAmp PCR kit (Perkin Elmer Cetus, Norwalk, Connecticut). A reaction volume of 100  $\mu$ l of PCR mixture contained 100 mM Tris-HCL (pH = 8.3), 500 mM KCl, 200  $\mu$ M each nucleotide [deoxyadenosine triphosphate (dATP), deoxycytidine triphosphate (dCTP), deoxyguanosin triphosphate (dGTP), deoxythymidine triphosphate (dTTP)] (Perkin Elmer), 0.9  $\mu$ M of each primer, 2.5 Units AmpliTaq DNA polymerase (Perkin Elmer Cetus), and 2.5 mM of MgCl. Samples were overlaid with 50  $\mu$ l of mineral oil to prevent evaporation. Polymerase chain reaction amplification was performed in a thermal cycler (PHC-2 Dri-Block; Techne, Princeton, New Jersey, USA). The cycling was programmed for denaturing at 94 C for 30 sec, annealing at 55 C for 40 sec, and extension at 74 C for 55 sec for a total of 35 cycles. The final extension was allowed to run for 5 min. Previously isolated spirochetal DNA was amplified with the synthesized primers. *Salmonella enteritidis* DNA was used as a negative control. Duplicate samples of the *B. burgdorferi* DNA were run in three concentrations (10 ng/100  $\mu$ l, 1 ng/100  $\mu$ l, 0.5 ng/100  $\mu$ l) and the *Salmonella*

*enteritidis* DNA at 10 ng/ $\mu$ l. Twenty-one samples of isolated tissue DNA, which included skin, kidney, heart, spleen, and liver, were amplified. Controls included tissue DNA of a non-infected birds, the *B. burgdorferi* DNA, and the DNA extracted from the chicken liver injected with the *B. burgdorferi*. HindIII digested lambda DNA (Sigma Chemical Company) was used as a size marker. The 1% agarose (BRL) gels were run in Tris-borate buffer (Sambrook et al., 1989) at 60 volts and run for 2 hr and stopped when the tracking dye reached the bottom of the gel. The gel was stained with ethidium bromide (1 $\mu$ g/ml) and washed three times with distilled water. The DNA products were seen by ultra-violet illumination at 300 nm and were photographed with a Polaroid camera.

For the use as DNA probe in slot blot DNA hybridization, 200 ng PCR amplified DNA product of *B. burgdorferi* was digested using 10 units of EcoRI in 20  $\mu$ l reaction buffer (Sambrook et al., 1989). The digested DNA was heat denatured (95 C, 5 min) and labeled with [ $\alpha$ - $^{32}$ P]-dATP using an end labeling kit from Am-bion Inc. (Austin, Texas, USA) according to the manufacturer's instructions. The 1  $\mu$ g amplified DNA from tissue samples were digested using EcoRI restriction enzyme (Sigma Chemical Company), followed by the addition of six times with a mixture of 0.9 M NaCl and 0.09 M sodium citrate (pH 7.0), (SSC), and denatured by boiling for 5 min. The samples were applied to a Zeta-Probe membrane (Bio-Rad) using the microfiltration unit (slot blot manifold) (Bethesda Research Lab) for binding nucleic acid solution on to a Zeta-Probe membrane (Bio-Rad). The membrane was vacuum dried and baked for 2 hr at 80 C, sealed inside a plastic bag with a prehybridization solution (1 mM EDTA, 0.5 M NaH<sub>2</sub>PO<sub>4</sub>, pH 7.2) and incubated for 5 min at 65 C to completely coat the membrane.

The labeled DNA probe was added with fresh buffer to the Zeta-Probe membranes and allowed to hybridize overnight by shaking at 65 C. The membrane was removed from the bag and washed at 65 C twice for 1 hr in a solution made up of 1 mM EDTA, 40 mM NaHPO<sub>4</sub>, pH 7.2 and 5% SDS. This was followed by two more washes at 65 C for 1 hr in a solution containing 1 mM EDTA, 40 mM NaHPO<sub>4</sub>, pH 7.2, and 1% SDS. The membranes were air dried and exposed to X-ray film for 6 hr using an intensifying screen at -70 C (Sambrook et al., 1989).

## RESULTS

Using the BSK culture, *B. burgdorferi* was isolated from the blood of one bird 3 wk post-infection. After weekly examinations of the blood inoculated media, *B.*

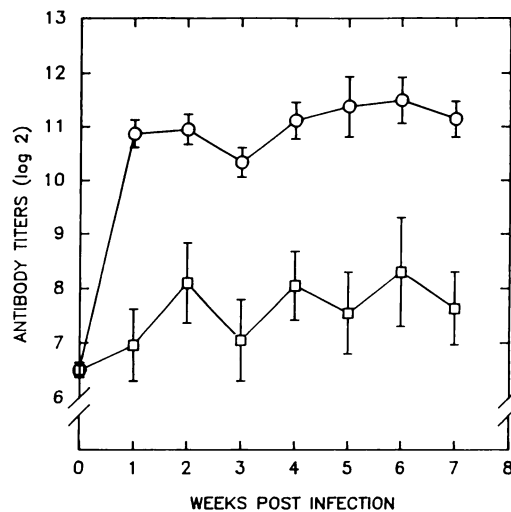


FIGURE 1. Geometric mean antibody response (ELISA) in *Borrelia burgdorferi* infected bobwhite quail.  $\square$ , uninfected controls;  $\circ$ , infected with *Borrelia burgdorferi*.

*burgdorferi* was identified in culture 6 wk post-inoculation. The remaining cultures from blood and tissues (kidney, liver, skin, heart and spleen) of the 24 infected and five non-infected birds were negative.

Based on the ELISA analysis, all 24 infected birds from which pre-infection titers had been established, developed antibodies by the end of 3 wk. The pre-infection titers for 21 of the birds was  $\leq$ 1:80 and for three birds the titers were 1:320. Ser-conversion was defined as a four-fold increase in antibody titer, which was not shown by any of the control birds. There was a dramatic rise in geometric mean titers in the first week post-infection, with a slight depression in the third week (Fig. 1). There was a second rise in titers by the fourth week, which remained elevated for the duration of the study period.

The bird sera were tested by the immunoblot method against both the *B. burgdorferi* strain used to infect the birds and the organism which was re-isolated from an infected host. The protein banding patterns of immune sera from infected birds tested against the protein from the two borrelia cultures were antigenically similar when comparing the bands most

commonly associated with Lyme disease, namely flagellin, OspA and OspB. The lower banding patterns differed, perhaps due to slight variations in conditions at the time of blotting or due to changes which occurred after the spirochete was passed through the bird (Fig. 2).

Five of 24 infected birds had focal hemorrhagic skin lesions at the injection sites and one of these birds had a similar lesion over the keel bone. All other organs in all the birds, infected and non-infected, appeared normal. Histopathologically, a diffuse inflammatory response, corresponding to the focal skin lesions described above, was seen on the H&E slide preparations of fixed skin tissue from these five birds. The lesions were hemorrhagic and had subcutaneous and dermal infiltration of plasma cells and lymphocytes. The Steiner stain slide preparations had a few fragmented borrelia in the skin sections.

In the direct FA test, borrelia were detected in the control preparations, which had been injected with live *B. burgdorferi*. No borrelia were seen on 80 sections of tissues from five non-infected birds. Of the 280 tissue sections examined from 14 infected birds, nine kidney sections from three birds were positive for borrelia.

With the PCR evaluation 21 tissues samples of kidney, liver, spleen, heart, and skin, from 14 infected birds yielded a 246 base pair (bp) DNA product as expected. Representative *B. burgdorferi* infected tissue samples indicating the amplified DNA product of 246 bp on 1% agarose gel are shown in Fig. 3. The slot blot hybridizations had specificity for detecting PCR amplified product from *B. burgdorferi* infected tissue (Fig. 4).

#### DISCUSSION

Northern bobwhite quail experimentally infected with *B. burgdorferi* did not develop evident clinical signs or lesions. The antibody response to *B. burgdorferi*, the isolation of spirochetes from the blood, the FA demonstration of borrelia in nine kidney sections, and PCR amplification of

specific DNA from the tissue of the infected birds confirmed the infection. An antibody response developed by the end of the first week post-infection and remained elevated for the 7-wk study period. However, there were no clinical signs of disease, gross or histopathologic lesions, except an inflammatory response at the injection site of five birds. These results were similar to the findings of earlier studies on mallard ducks (*Anus platyrhynchos*) and wild turkeys (*Meleagris gallopavo*) which did not develop clinical signs but had positive cultures and antibody titers (Burgess, 1989). *Borrelia burgdorferi* was isolated from the liver of a veery (*Catharus fuscescens*) (Anderson et al., 1986) and it was later shown that larvae of *Ixodes scapularis* (formerly *I. dammini*), which had fed on birds, were infected with the borrelia (Anderson, 1988; Anderson et al., 1990; McLean et al., 1993).

While PCR results confirmed DNA in all tissues tested, the FA test showed that only a few intact spirochetes may have been present. Culture of *B. burgdorferi* from tissues resulted in the recovery of spirochetes from the blood of only one bird. The re-isolation of borrelia from the blood at 3 wk post-infection was ascertained to be the same organism as the original culture by immunoblot analysis (Fig. 2).

Based on the presence of antibodies, as detected by ELISA, there appeared to be an active infection in the experimentally inoculated birds. Sixteen of 24 infected birds developed antibodies within 1 to 3 wk post-infection, and later on all of the infected birds had antibodies. None of the control birds developed antibodies. The persistence of elevated titers throughout the 8-wk study period could suggest either an active infection or the persistence of *B. burgdorferi* antigens, as is seen in post antibiotic treatment of both human and animals with borrelia infection (Golightly et al., 1990; Appel, 1990). The immunoblot tests support the ELISA data. The sera of the three infected birds on which immunoblot tests were done reacted to proteins

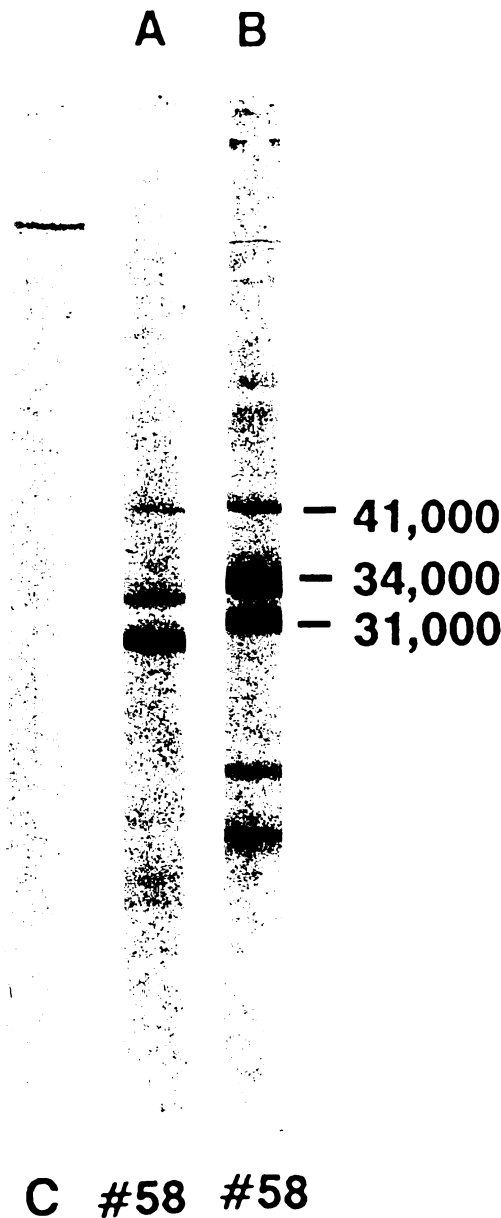


FIGURE 2. Western immunoblot analysis showing antibody reaction of sera of one bobwhite quail against *Borrelia burgdorferi* cultures. A. Culture used to infect bird; B. culture recovered from infected bird; C. control. Molecular-mass values are given in daltons.

specific to *B. burgdorferi*. The bands detected in the immunoblots, corresponded to bands previously determined to be associated with *B. burgdorferi*, especially flagellin (41Kd), Osp A, and Osp B (Craft

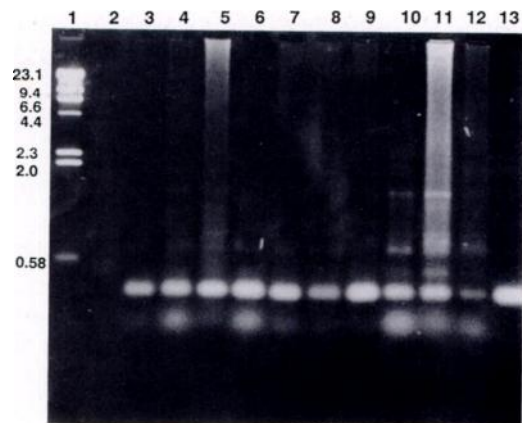


FIGURE 3. Representative agarose gel electrophoresis showing polymerase chain reaction amplified *Borrelia burgdorferi* DNA from tissues of experimentally infected bobwhite quail. Lane 1: Hind III digested lambda DNA marker; 2: kidney (non-infected bird); 3 and 4: skin from bird 61; 5: kidney from bird 80; 6 and 7: kidney from bird 81; 7 and 8: heart from bird 81; 9: kidney from bird 82; 10: liver from bird 78; 11: spleen from bird 77; 12: kidney from bird 77; and 13: liver injected with *Borrelia burgdorferi* used as a positive control.

et al., 1986; Schwan et al., 1989; Zoller et al., 1991).

The PCR amplification showed *B. burgdorferi* DNA in several tissues and indicated the presence of a disseminated in-

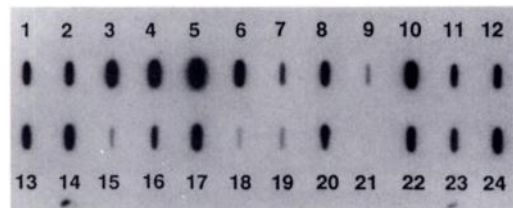


FIGURE 4. Slot blot hybridization assay using amplified DNA product specific for *Borrelia burgdorferi* as a probe. Lane 1: skin from bird 58; 2: kidney from bird 82; 3: kidney from bird 81; 4: heart from bird 81; 5: kidney from bird 80; 6: spleen from bird 80; 7: liver from bird 78; 8: spleen from bird 77; 9: kidney from bird 74; 10: heart from bird 71; 11: kidney from bird 69; 12: kidney from bird 67; 13: kidney from bird 66; 14: heart from bird 63; 15: kidney from bird 63; 16: spleen from bird 61; 17: spleen from bird 58; 18: kidney from bird 58; 19: spleen from bird 49; 20: kidney from bird 49; 21: kidney from bird 46; 22 to 24: chicken liver injected with *Borrelia burgdorferi* as a positive controls.

fection. The amplified DNA was confirmed by the slot blot DNA hybridizations. The results were consistent with similar studies done by others in which *B. burgdorferi* was detected in the similar tissue specimens from infected gerbils (*Meriones unguiculatus*) (Lebeche et al., 1991; Wise and Weaver, 1991) and by Goodman et al. (1991) in which the borrelia was identified in the urine of human patients with the disease.

The presence of the DNA was not necessarily evidence of an active infection as the DNA of dying or dead spirochetes would be detectable by PCR. Based on the recovery of the *B. burgdorferi* at 3 wk post-infection and the presence of FA-positive borrelia in the viscera, we believe that the borrelia were able to survive for that period of time, and that birds are susceptible to infection by *B. burgdorferi*. Thus, the birds ability to carry ticks and to infect ticks in new areas makes them potentially important in the spread of this disease. Migrating birds feed in different regions along their flyways, a factor which increases the possibility of either disseminating already infected ticks or of infecting local ticks present in non-endemic areas. Because *Ixodes scapularis* remains attached to the host for a period of 2 to 6 days (Piesman et al., 1991), the dispersion of ticks could be widespread. At the time of the northern migration in the spring, nymphal activity has begun and any infected immature ticks have maintained spirochetes over the winter (Burgdorfer, 1984). The potential exists that migrating birds transport infected ticks, or carry the spirochete, or both. In the northeast, larval feeding period begins in August and extends into the fall. This time period coincides with the fall migration of birds, which would allow the dispersion of ticks southward from endemic areas.

In order to determine the frequency with which birds may disseminate the infection, additional research is required to show both the duration of active avian borreliosis and the extent to which the spirochetes replicate in birds. While this information would

be important, findings of a short infection period would not dramatically lessen the bird's role.

#### LITERATURE CITED

- ANDERSON, J. F. 1988. Mammalian and Avian reservoirs for *Borrelia burgdorferi*. *Annals of the New York Academy of Sciences* 539: 180-191.
- , R. C. JOHNSON, L. A. MAGNARELLI, AND F. W. HYDE. 1986. Involvement of birds in the epidemiology of the Lyme disease agent *Borrelia burgdorferi*. *Infection and Immunity* 51: 394-396.
- , L. A. MAGNARELLI, AND K. STAFFORD III. 1990. Bird-feeding ticks transtadially transmit *Borrelia burgdorferi* that infect Syrian hamsters. *Journal of Wildlife Diseases* 26: 1-10.
- APPEL, M. J. 1990. Lyme disease in dogs and cats. *Compendium on Continuing Education for the Practicing Veterinarian* 12: 617-625.
- BARBOUR, A. G. 1984. Isolation and cultivation of Lyme disease spirochetes. *The Yale Journal of Biology and Medicine* 57: 521-525.
- BEAUCAGE, S. L., AND M. H. CARUTHERS. 1981. Deoxynucleotide phosphoramidites: A new class of key intermediates for deoxypolynucleotide synthesis. *Tetrahedron Letter* 22: 1859-1862.
- BURGDORFER, W. 1984. Discovery of the Lyme disease spirochetes and its relation to tick vectors. *The Yale Journal of Biology and Medicine* 57: 515-520.
- BURGESS, E. 1989. Experimental inoculation of mallard ducks with *Borrelia burgdorferi*. *Journal of Wildlife Diseases* 25: 99-102.
- CRAFT, J. E., D. K. FISCHER, G. T. SHIMAMOTO, AND A. C. STEERE. 1986. Antigens of *Borrelia burgdorferi* recognized during Lyme disease. *Journal of Clinical Investigation* 78: 934-939.
- GOLIGHTLY, M. G., J. A. THOMAS, AND A. L. VICIANA. 1990. The laboratory diagnosis of Lyme disease. *Laboratory Medicine* 21: 299-304.
- GOODMAN, J. L., P. JURKOVICH, J. M. KRAMER, AND R. C. JOHNSON. 1991. Molecular detection of persistent *Borrelia burgdorferi* in the urine of patients with active Lyme disease. *Infection and Immunity* 59: 269-278.
- HRAPCHAK, R. J. 1980. Immunochemistry. *In Theory and practice of histotechnology* edits, D. Sheehan and B. B. Hrapchak (eds.). Battelle Press, Columbus, Ohio, pp. 143-144, pp. 314-315.
- LAEMMLI, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680-685.
- LEBECH, A., P. HINDERSON, J. VUUST, AND K. HANSEN. 1991. Comparison of in vitro culture and polymerase chain reaction for the detection of *Borrelia burgdorferi* in tissues from experimentally infected animals. *Journal of Clinical Microbiology* 29: 731-736.



- MAGNARELLI, L. A., J. M. MEGAN, J. F. ANDERSON, AND A. CHAPPELLE. 1984. Comparison of an indirect fluorescent-antibody test with an enzyme-linked immunosorbent assay for serological studies of Lyme disease. *Journal of Clinical Microbiology* 20: 181–184.
- MALLOY, D. C., R. K. NAUMAN, AND H. PAXTON. 1990. Detection of *Borrelia burgdorferi* using the polymerase chain reaction. *Journal of Clinical Microbiology* 28: 1089–1093.
- MCLEAN, R. G., S. R. UBICO, C. A. NORTON HUGHES, S. M. ENGSTROM, AND R. C. JOHNSON. 1993. Isolation and characterization of *Borrelia burgdorferi* from blood of a bird captured in the Saint Croix River Valley. *Journal of Clinical Microbiology* 31: 2038–2043.
- PIESMAN, J., G. O. MANPIN, E. G. CAMPOS, AND C. M. HAPP. 1991. Duration of adult female *Ixodes dammini* attachment and transmission of *Borrelia burgdorferi*, with description of a needle aspiration method. *The Journal of Infectious Diseases* 163: 895–897.
- ROGERS, B. B., L. C. ALPERT, E. HINE, AND G. BUFFONE. 1990. Analysis of DNA in fresh and fixed tissue by the polymerase chain reaction. *American Journal of Pathology* 136: 541–548.
- SAMBROOK, J., E. FRITSH, AND T. MANIATOUS. 1989. Molecular cloning. Cold Harbor Laboratory. Cold Harbor Spring, New York, pp. 1.25–1.38, 5.28–5.31, 6.3–6.45.
- SCHWAN, T. G., K. KIME, M. E. SCHUMPF, J. E. COE, AND W. SIMPSON. 1989. Antibody response in white-footed mice (*Peromyscus leucopus*) experimentally infected with the Lyme disease spirochete (*Borrelia burgdorferi*). *Infection and Immunity* 57: 3445–3451.
- SCHWARTZ, R. 1987. Common black-headed gull from Holland. *Connecticut Warbler* 7: 21.
- SWISHER, B. L. 1987. Modified Steiner procedure for microbiologic staining of spirochetes and non-filamentous bacteria. *Journal of Histotechnology* 10: 241–243.
- TOWBIN, H., T. STAHELIN, AND J. GORDON. 1979. Electrophoretic transfer of proteins from SDS gel and acid/urea gels to nitrocellulose membranes. *Proceeding of National Academy of Science USA* 76: 4350–4354.
- WISE, D. J., AND T. L. WEAVER. 1991. Detection of the Lyme disease bacterium, *Borrelia burgdorferi* by using the polymerase chain reaction and a non-radioisotopic gene probe. *Journal of Clinical Microbiology* 29: 1523–1526.
- ZOLLER, L., S. BURKARD, AND H. SCHAFER. 1991. Validity of western immunoblot band patterns in the serodiagnosis of Lyme borreliosis. *Journal of Clinical Microbiology* 29: 174–182.

*Received for publication 30 July 1993.*