Zbl. Bakt. Hyg. A 263, 189–200 (1986)

Human Fetal Borreliosis, Toxemia of Pregnancy, and Fetal Death

ALAN B. MACDONALD

Southampton Hospital, Long Island, New York, U.S.A.

Introduction

The potential for transplacental infection of the human fetus is recognized for syphilis, leptospirosis, and relapsing fever borreliosis. A case of maternal – fetal transmission of the Lyme disease spirochete, *Borrelia burgdorferi,* has recently been reported (1). This report describes four cases of fetal borreliosis which were encountered in a prospective study of abortuses.

Clinical Case Summaries

Case 1: A 24 year old woman (G 1, P 0, Ab 0) delivered a 2500 gram stillborn male fetus at term. Her prenatal care record was negative for factors associated with high risk pregnancy. No infections were diagnosed during the prenatal period.

Case 2: A 22 year old woman (G 1, P 0, Ab 0) delivered a 514 gram stillborn macerated female fetus at nineteen weeks gestation. Her antepartum course was complicated by toxemia of pregnancy which had its onset during the seventeenth week of pregnancy and was manifest by hypertension, albuminuria, facial edema, and peripheral edema. No infections wer diagnosed during the prenatal period.

Case 3: A 37 year old woman (G 3, P 0, Ab 2) delivered a 490 gram stillborn male fetus showing mild maceration at twenty three weeks gestation. She had a chronic collagen disease in clinical remission during the pregnancy. The collagen disease was marked by episodes of fever, thrombocytopenia, anemia, arthritis, and lymphadenopathy and had been controlled with low dose oral prednisone. The patient was not receiving prednisone during the antepartum period. Amniocentesis was performed at the twentieth week of gestation and revealed a normal fetal karyotype. Toxemia of pregnancy had its onset during the twenty second week of gestation and was manifest as hypertension, and proteinuria. No infections were diagnosed during the prenatal period.

Case 4: A 32 year old woman (G 2, P 0, Ab 1) delivered an 85 gram female fetus showing mild maceration at 15 weeks gestation. Her antepartum clinical record revealed no factors for a high risk pregnancy. No infections were diagnosed during the antepartum period.

Methods

Culture of Autopsy Tissue: Flame sterilized surgical instruments were used to transfer fresh tissue from the fetal cadaver to sterile modified Kelly's medium. Tissue volumes ranged from 0.5 ml to 1.5 ml. The volume of modified Kelly's medium was 10 ml. The specimens were incubated at 32 degrees C. Small aliquots were removed at weekly intervals using sterile technique and

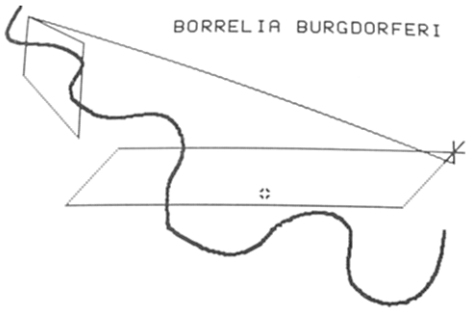

Fig. 1. *Borrelia burgdorferi* in schematic design with potential configurations of portions of the spirochete in tissue sections

examined by darkfield microscopy. Subcultures were initiated when any of the specimens showed either overgrowth of nonspirochetal bacterial flora or showed spirochetes by darkfield microscopy.

Preparation of Agar Controls: Spirochetes were harvested from the log phase of growth of the B31 reference strain of *Borrelia burgdorferi,* were washed three times in sterile phosphate buffered saline 0.9 normal saline solution, pH 7.4 with centrifugation of the spirochetes at 10 degrees C. between each wash step. The spirochetes were than fixed with 10 percent formalin solution in buffered saline solution pH = 7.4. A small aliquot was air dried on a glass slide and tested for reactivity against pooled patient serum known to be reactive against *B. burgdorferi* using an indirect immunofluorescence assay with fluorescein conjugated goat anti human IgG (Kallestadt laboratories). The formalin fixed spirochetes were centrifuged to a pellet and the pellet was transferred to a semisolid preparation of brain heart infusion agar in 0.9 percent phosphate buffered saline. The agar was allowed to gel at room temperature, and the agar-spirochete matrix was fixed in 10 percent buffered formalin, sectioned into 3 mm wafers, and processed for tissue sections with serial solutions of alcohols, xylenes, and paraffin embedding. Paraffin sections 5

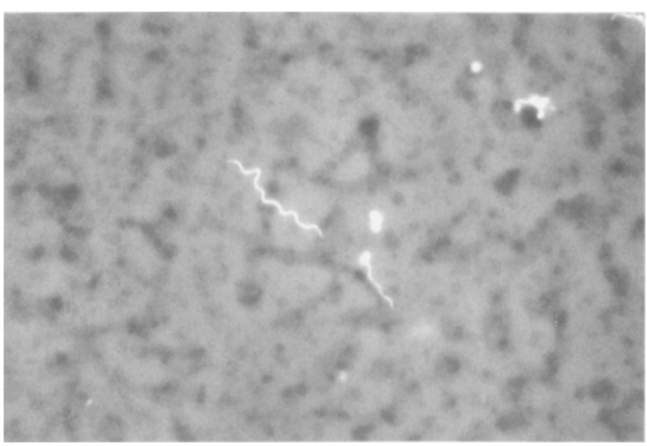

Fig. 2. *Borrelia burgdorferi,* reference strain B 31 (Magnification 1000 ×, acridine orange)

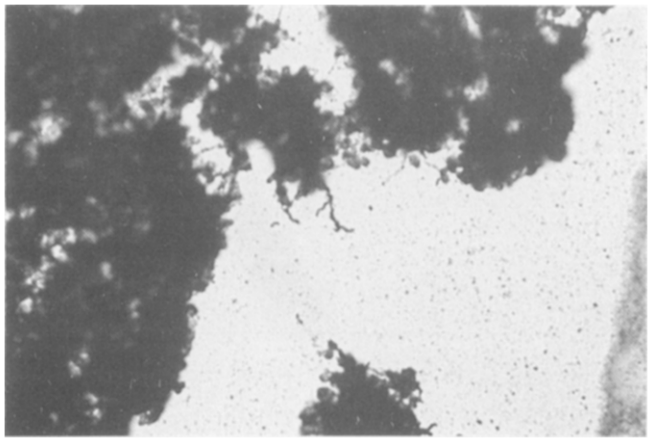

Fig. 3. *Borrelia burgdorferi*, strain B 31, in 5 micron tissue section from agar control (magnification 1000 ×, Warthin-Starry stain)

Fig. 4. *Borrelia burgdorferi*, in tissue section from agar control (magnification 1000 ×, Warthin-Starry stain)

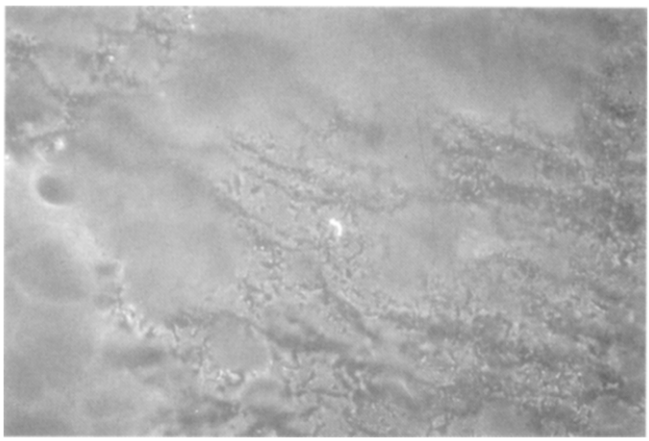

Fig. 5. *Borrelia burgdorferi*, in tissue section from agar control (magnification 1000 ×, IFA, polyclonal human serum reactive against B 31 strain of *Borrelia burgdorferi*)

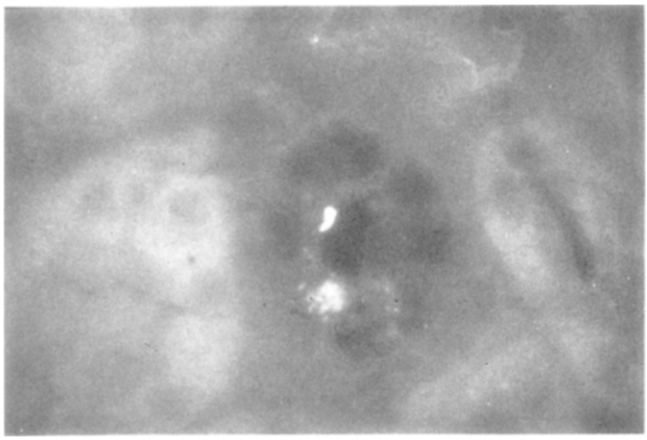

Fig. 6. *Borrelia burgdorferi* in hamster kidney (magnification 1000 ×, IFA, polyclonal human serum)

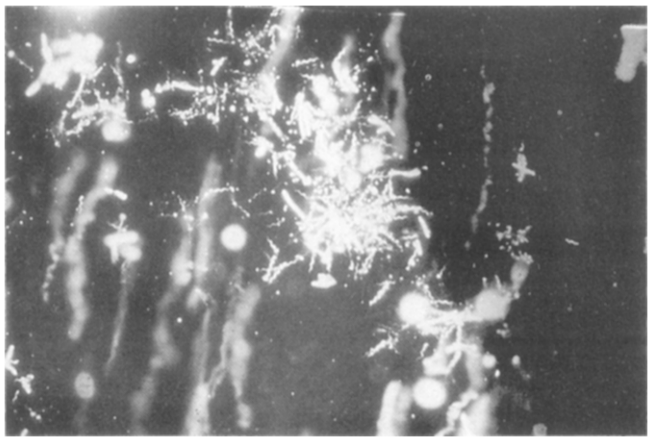

Fig. 7 *Borrelia burgdorferi* in BSK culture from autopsy fetal liver, case one, with Bacillus species contaminant (magnification 400 × darkfield)

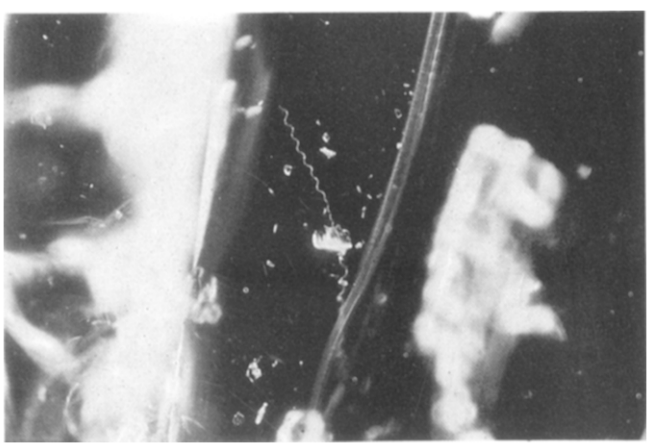

Fig. 8. *Borrelia burgdorferi*, in subculture from isolate of figure 7 (magnification 400 ×, darkfield)

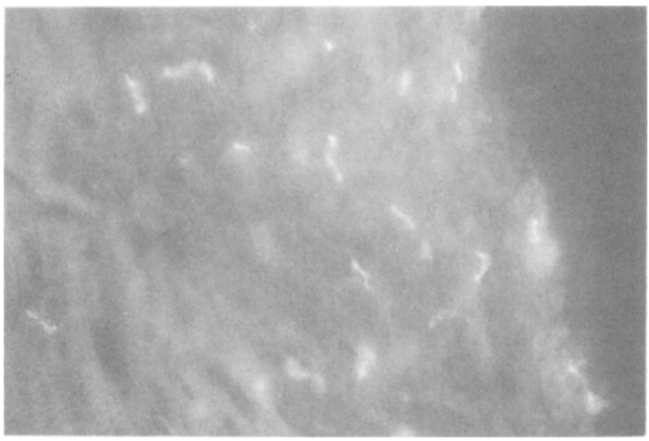

Fig. 9. *Borrelia burgdorferi* in fetal myocardium, case one (magnification 1000 ×, IFA, polyclonal human serum)

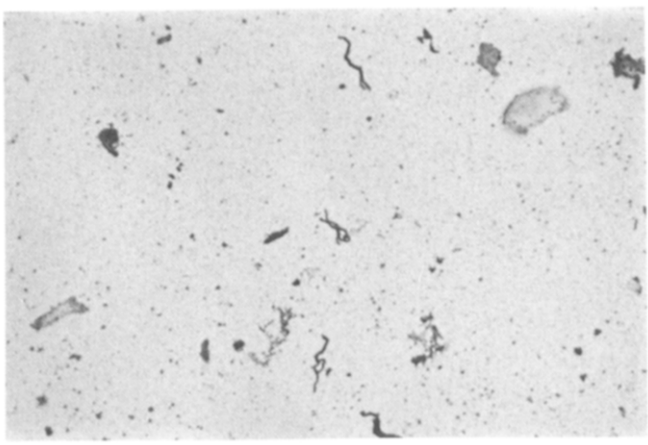

Fig. 10. *Borrelia burgdorferi* in arachnoid space of fetal midbrain, case one (magnification 1000 ×, Warthin-Starry stain)

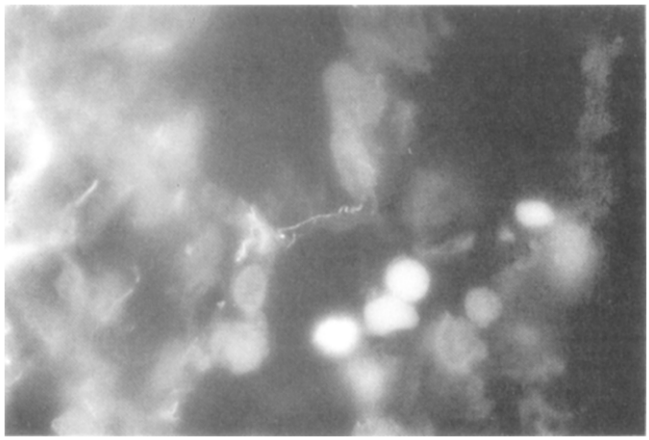

Fig. 11. *Borrelia burgdorferi* in arachnoid space of fetal midbrain case one (magnification 1000 ×, IFA, monoclonal antibody from Dr. Alan Barbour, Rocky Mountain Laboratory, Hamilton, Montana)

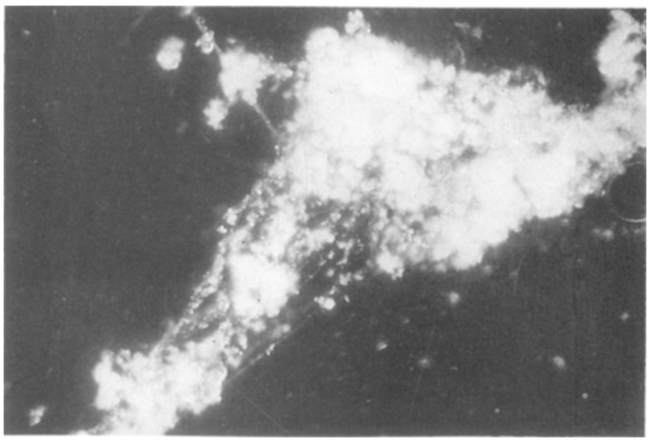

Fig. 12. *Borrelia* species and contaminant bacterial species from culture of fetal liver, case 2 (magnification 400 ×, darkfield)

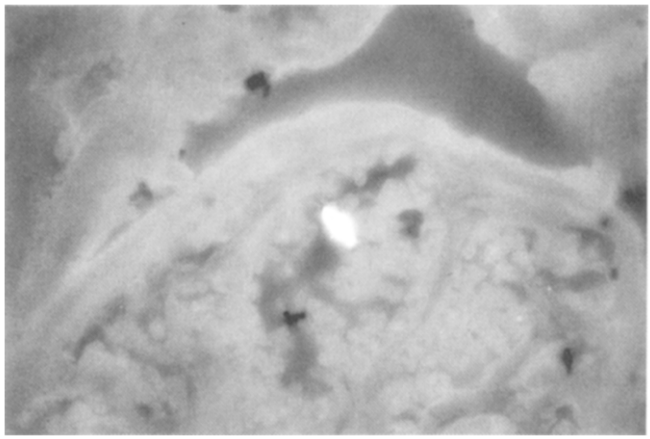

Fig. 13. *Borrelia* species in placental villus, case 2 (magnification 1000 ×, IFA, polyclonal human serum)

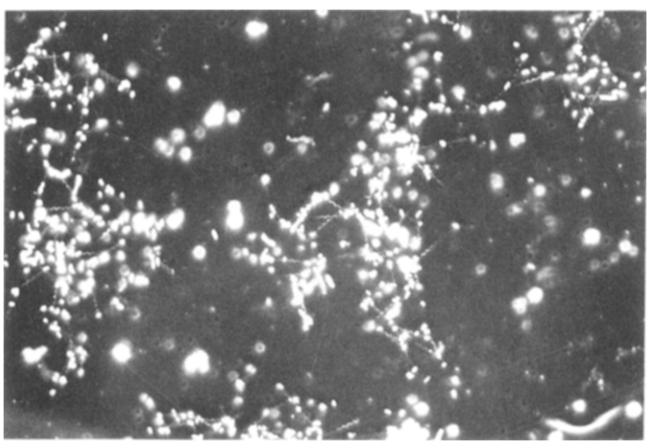

Fig. 14. *Borrelia* species from BSK culture of fetal liver, case 3 (magnification 400 ×, darkfield)

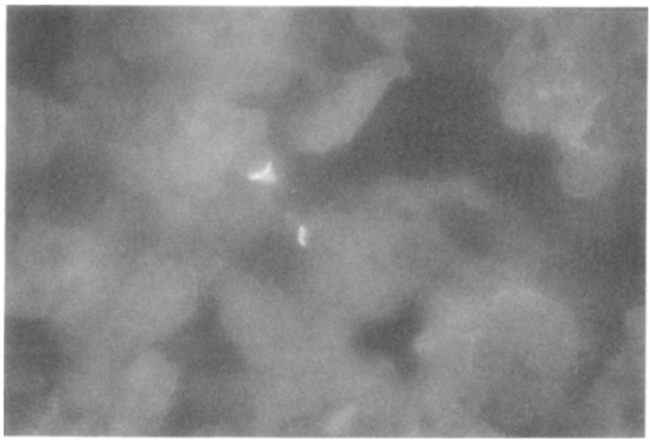

Fig. 15. *Borrelia* species in fetal liver, case 3 (magnification 1000 ×, IFA, polyclonal human serum)

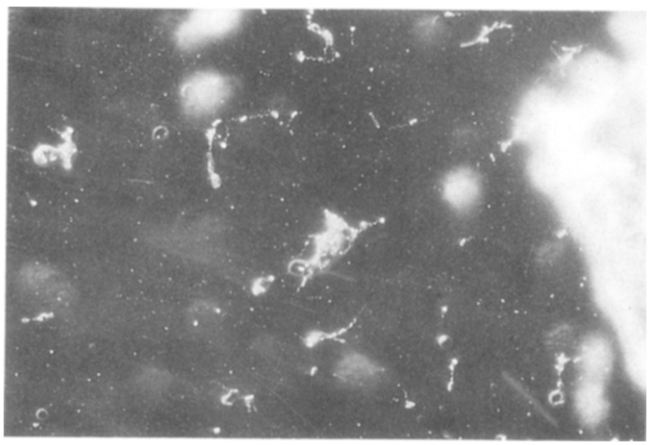

Fig. 16. *Borrelia* species from culture of fetal liver, case four (magnification 400 ×, darkfield)

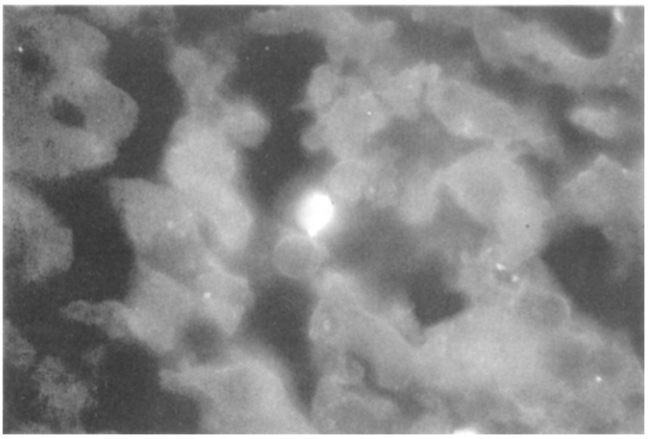

Fig. 17. *Borrelia* species in fetal liver, case four (magnification 1000 ×, IFA, polyclonal human serum)

CULTURE OF BORRELIA
FROM FETAL TISSUES AT AUTOPSY

AUTOPSY USING SEMI-STERILE TECHNIQUE
TISSUES OBTAINED WITH FLAME STERILIZED SURGICAL INSTRUMENTS
TISSUES TRANSFERED TO MODIFIED KELLY'S MEDIUM
INCUBATION AT 32 DEGREES CENTIGRADE
DARKFIELD EXAMINATION OF CULTURES AT WEEKLY INTERVALS
DIFFERENTIAL CENTRIFUGATION TO CONCENTRATE BORRELIA
SUBCULTURE OF POSITIVE CULTURES
MONOCLONAL ANTIBODY IFA ON POSITIVE CULTURES

Fig. 18. method

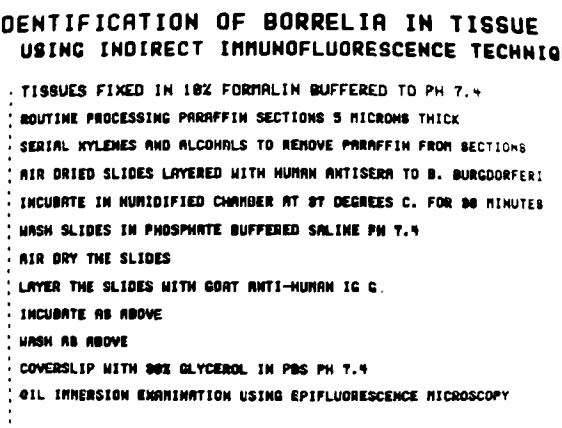

Fig. 19. method

microns thick were prepared, the paraffin was removed with graded xylenes and alcohol solutions, and the slides were air dried.

Indirect Immunofluorescence on Paraffin Sections: After removal of paraffin from 5 micron tissue sections with graded xylene and alcohols, the sections were allowed to air dry. They were successively layered with pooled human serum known to be reactive against *B. burgdorferi,* washed in sterile phosphate buffered saline pH 7.4, air dried, and layered with goat anti-human IgG conjugated to fluorescein (Kallestadt Laboratories), washed, and examined using epifluorescence microscopy under 1000 × magnification.

Silver Stains on Paraffin Sections: Deparaffinized sections were impregnated with a one percent solution of silver nitrate in deionized water pH 7.4 or 30 minutes at 56 degrees centigrade, and were differentiated with a solution of 2 percent silver nitrate, gelatin, and hydroquinone in deionized water under direct visual control until the tissue sections assumed a golden yellow brown color. (Warthin-Starry Method).

Results

Culture of Fetal Tissues:

Spirochetes were cultured from fetal liver tissue in each of the four cases. The time varied for a positive culture to be detected from 10 days to 6 months. Spirochetes were cultured from the fetal heart in case one. Aggregates of spirochetes resembling brood colonies were found in slow growing cultures. Doublets of spirochetes fused at an apical pole were found in fast growing specimens. The length of the spirochetes increased in serial observations of positive primary cultures, and after several months of observation, the spirochetes tended to form brood colonies. The cultures were contaminated in all cases, but in spite of contamination, the spirochetes continued to grow.

Immunofluorescence of Tissue Sections:

Spirochetes were detected in fetal liver, heart, adrenal, brain, kidney, meninges, and in the subarachnoid space in case number one. Limited examination of tissues from the remaining cases revealed spirochetes in fetal liver, or placents, although the involvement of other organ systems was not investigated. Spirochetes were reactive against the

pooled human serum lot described above in all cases, and against a monoclonal mouse antibody specific for *B. burgdorferi* in case number one. (Monoclonal antibody obtained from Dr. *Alan Barbour,* Rocky Mountain Laboratory, Hamilton, Montana).

Immunofluorescence of Positive Culture Specimens:

The isolate of spirochetes from the liver of the fetus in case number one was concentrated with centrifugation, washed in buffered cold saline solution, and air dried on a glass slide. After fixation in alcohol, the concentrate was layered with the mouse monoclonal antibody to *B. burgdorferi,* followed by fluorescein conjugate anti-mouse IgG. A positive fluorescence was detected on the spirochetes from the culture.

Discussion

Spirochetes were cultured from fetal liver in four stillborn human fetuses, three of whom demonstrated congenital malformations of the heart or great vessels (Ventricular septal defect – 1 case, atrial septal defect – 1 case, coarctation of the aorta – 1 case). Toxemia of pregnancy was found in two of the cases in the series. Spirochetes were identified in paraffin embedded formalin fixed fetal tissues in each case in this series using a simple indirect immunofluorescent microscopic method. A tissue equivalent was developed to study the variations of morphology of *B. burgdorferi* in 5 micron tissue sections using both the Warthin-Starry silver stain and a pooled human anti-*Borrelia burgdorferi* serum immunofluorescent method. The IFA method was successfully extended to mouse monoclonal antibody to *B. burgdorferi* as an antigen probe. The tissue IFA method can be applied to the retrospective and prospective study of the possible role of *B. burgdorferi* as the etiologic agent of fetal demise of uncertain cause, congenital heart defects, and miscarriage following maternal toxemia of pregnancy.

Reference

1. *Schlesinger, P. A., P. H. Duray, B. A. Burke, A. C. Steere, T. Stillman:* Maternal-Fetal transmission of the Lyme disease spirochete, Borrelia burgdorferi. Ann. Int. Med. 103 (1985) 76–78

Alan B. MacDonald, M. D., Southampton Hospital, Long Island, New York, U.S.A.