Dear Dr. Med. Marie-Cecile Ploy,

I congratulate you on your very important case report of
borrelia endocarditis.
Superb!
The DNA evaluation makes the role of B. Afzelii indisputable.

We will report our research on biofilms of Borrelia burgdorferi in vitro
findings in PLOS ONE to be released on their website tomorrow.
[Full and complete open access]

As an anatomic pathologist, my interest in borrelia pathobiology
goes back 30years,with much time spent finding Borrelia burgdorferi
in human tissues and in mammalian tissues. I used the Warthin Starry
stain 30 years ago, but abandoned it for Immunohistochemistry
using high titer human ploy clonal antibodies,[initial studies] and
later Monoclonal murine antibodies (h5332 h9724) which are
specific for the North american B31 strain of Bb ss.
In my studies, it became clear that in human tissue, and under conditions
of adverse growth conditions, the spiral form of Borrelia is lost.
It is replaced by various forms (shape shifted forms) including, but not
limited to :Straightened forms, bacilliform or vibrio-form like , membrane duplication
forms, ring forms, granular forms, cystic forms, and cell wall deficient forms.
I attach two PDF files which will illustrate this morphologic diversity,
especially in the biofilm communities of borrelia burgdorferi.

I would not reject, out of hand that the curved nonspiral form, which you saw in Warthin Starry staining of your vegetation, might be a completely valid form of Bb [attachments provided]

We now have DNA probes and in situ DNA hybridization methods to apply to human tissue for Bb strain B31. Unfortunately we do not possess DNA probes for B. Afzelii. I would recommend the Invitrogen products for making your DNA primer sets to function as fluorescent labelled DNA probes in In Situ DNA hybridization in your heart valve vegetation.

Such In situ hybridization would add a level of specificity to your elegant studies in your case report.

I would be pleased to provide a procedure for your lab to accomplish these studies. I routinely demand that a paraffin embedded borrelia spirochetal control be run in parallel with any histological staining procedure to detect any pathogen in tissue.

For borrelia Afzelii, I would recommend: BSK cultures of B Afz. are allowed to "age" so that the motile forms diminish and the medium Ph indicator turns yellow. Add a 2cc aliquot of the aged culture to sterile plasma (human or otherwise) filter sterilized through a 0.2 micron filter. Add topical thrombin to "clot" the mixture of aged Bb and plasma. The clot will form immediately. The clot will exert "shearing " forces across the axis of the spirochetes which recapitulate the shear in forces as spirochetes move through body tissues and Fibrin Clots. Place the Clotted fibrin with spirochetes into a standard vial with 10% neutral buffered formalin.

Fix overnight. then process the clot/spirochete mixture on a routine tissue processor in any pathology lab for preparation of paraffin slides.

De-paraffinize the slides carefully with extra time in the oven to anneal to the glass slide and extra time in serial xylenes to remove ALL traces of Wax. Take these to Water.

These slides may be stored in a dry state indefinitely at room temperature, in a dust proected slide container.

When the hybridization is performed, handle the Clot/spirochete positive controls Exactly the Same way that you handle your Heart valve vegetation paraffin slides [completely and rigorously dewaxed]

Please spend some time inspecting the Biofilms of borrelia burgdorferi which were prepared from pure Planktonic Borrelia obtained from the American Type Culture reference collection.

Please remark that most biofilms of borrelia burgdorferi have few or completely absent spiral forms.

I look forward to hearing from you,

With kind regards

and

Les sentiments les plus amicales,

Alan B. MacDonald MD,FCAP,FASCP