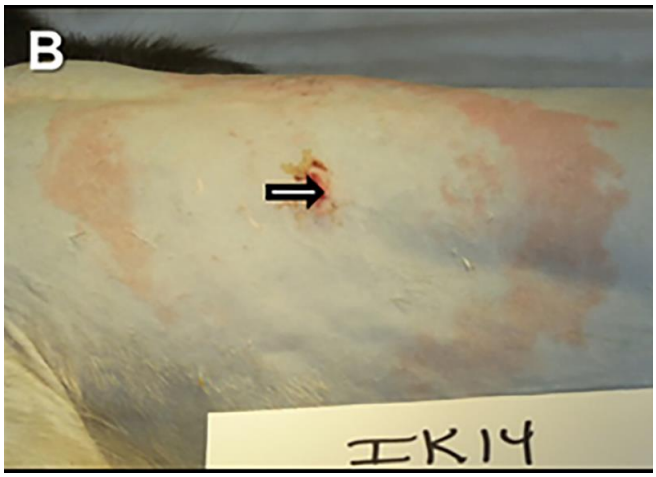
Vector-Borne Infection
Research-Analysis-Strategy

December 2017

Notes on:

Embers ME, Hasenkampf NR, Jacobs MB, Tardo AC, Doyle-Meyers LA, Philipp MT, et al. (2017) **Variable manifestations, diverse seroreactivity and post-treatment persistence in non-human primates exposed to Borrelia burgdorferi (B.b) by tick feeding**. PLoS ONE 12(12): e0189071. https://doi.org/10.1371/journal.pone.0189071

The meticulous experiment conducted by Embers *et al* has shown conclusively that Lyme disease infection can persist after moderately delayed, standard treatment. This has major implications for patients and physicians, who need to understand and use this information to make appropriate decisions about Lyme disease.



Embers *et al* investigated Lyme disease infection and treatment in ten macaque monkeys. The progression of the disease and the organs affected in these animals, closely matches that found in humans. The monkeys were infected with *borrelia burgdorferi* Lyme disease spirochaetes by at least ten bites of infected ticks. Only one of the monkeys developed a distinctive EM rash (photo). If this was representative, this would give an EM rash frequency of only 10%. At 16 weeks after infection, five of the monkeys were treated with the antibiotic doxycycline for four weeks, at a dosage which produced target blood levels matching those for the treatment of human Lyme disease.

The experiment could be interpreted as representing a scenario in which a Lyme disease patient: 1/ had a substantial initial infection, 2/ experienced a moderate delay in diagnosis of four months and, 3/ then received relatively aggressive antibiotic treatment sustained for four weeks.

The antibiotic treatment given to the monkeys matched or exceeded recommendations for the treatment of Lyme disease made by such authorities as Public Health England (PHE), the British Infection Association (BIA), and the National Institute of Clinical and Care Excellence (NICE).

Treatment did not eradicate the infection in any of the five monkeys.

>Xenodiagnosis: is a test by which the presence of a vector-borne infection can be confirmed by allowing the vector (which with Lyme disease is a tick), to feed on

a suspected infected subject. The tick can then tested for the presence of the infection by various methods such as antibody staining, PCR, etc.

The experiments reported by Embers *et al* show that all ten monkeys still had evidence of infection as shown by xenodiagnosis at either 3 months or 6-7 months after treatment. In four of the monkeys (two treated and two untreated), xenodiagnosis was the only method that demonstrated a sustained presence of B.b. However, in all four of these subjects, the xenodiagnosis was confirmed by at least two distinct methods, including PCR for a specific B.b protein sequence.

These results show that it is highly desirable that experiments aimed at evaluating a testing methodology or treatment efficacy should include xenodiagnostic methods. Lyme and suspected Lyme patients should be canvassed to assess the acceptability of this technique for furthering understanding of the disease in humans.

Antibodies monitored in the experiment

Embers et al employed a sophisticated technique based on fluorescence flow cytometry to measure the levels of specific anti-borrelia antibodies in the monkeys. These were checked at regular intervals throughout the trial. The five antibodies targeted were: OspA (p31), DbpA (decorin binding protein), OppA-2 (oligopeptide permease), OspC (p25) and C6 (VlSE). An excellent set of charts provided with the report and reproduced below, illustrate individual antibody levels measured throughout the experiment for each subject. These are in sufficient detail to permit a number of observations to be made:

(Please note that one of the treated monkeys (IK14) produced anomalous results. This was the only monkey to produce a distinct EM rash, yet it never produced any measurable antibodies for the 5 proteins studied. If this small monkey cohort was representative of human subjects, it raises the possibility that one in ten infected people tested would remain seronegative at all stages of infection.)

1. None of the animals produced antibodies to OspA. The authors suggest this could be due to transmission by tick inoculation, which represents the normal infection route in humans. It is possible that because the ticks used for transmission, were capillary fed with a laboratory culture of B.b, that the spirochaetes missed some protein expression stimuli prompted by the changing tick-gut/hemolymph/saliva environments prior to entering the mammal. Infection could have been via regurgitation from the tick gut rather than from pre-infection of the tick saliva.
2. By week 4 after infection, all the monkeys produced detectable levels of antibodies. At this time point, the levels for two or more antibodies in 7 out of 9 subjects, was close to the maximum levels detected for that subject throughout the experiment.
3. Antibody levels could rise and fall rapidly. In two to four weeks, DbpA, OppA-2 and OspC levels could change up or down by a substantial proportion of their maximum measured levels for a subject. E.g., in animal IP55 between weeks 30 to 34, the levels of three antibodies dropped by 20% to >50%.
4. DbpA appeared quickly and had the highest sustained detection levels. The levels fluctuated rapidly and sufficiently to show that sustained levels were not remnants of an 'old' response.

5. OppA-2 levels were often initially similar to DbpA, though in four treated and two untreated subjects, levels of OppA-2 started to drop down at around 16 to 22 weeks post-infection and then tended to remain lower. (Note, treatment was started at 16 weeks post-infection)
6. C6 showed the weakest response and lowest responsiveness and levels were almost always substantially lower than DbpA and OppA-2. The C6 response was strong only with monkey, IP55. IP67 had a very weak (seronegative) C6 response. With 6 animals a relatively weak C6 response started to decline at 18 weeks after infection. At time points ranging from ~30 to 40 weeks after infection, the Mean Fluorescence Intensity was less than 5% of the maximum value recorded. This data has implications for testing humans for Lyme disease, suggesting that 10% of patients could test false-negative after 4 weeks when an immune response is expected and up to 70% will be false-negative if testing has been delayed by 30 to 40 weeks after infection.
7. Declining levels of C6 antibodies over time, did not correlate with other antibody levels, even though the visual pattern was sometimes similar. Unlike C6, other antibodies often remained at seropositive levels throughout the experiment. The decline in C6 antibodies is particularly noticeable in the treated group suggesting that patients who have had any antibiotics should not be tested with a C6 only test.
8. It has long been known that antibody levels are not strongly associated with either the presence or level of a Lyme infection. The Embers et al data demonstrates that in most animals the initial infection was associated with rapid increase in antibody levels. Beyond this, changes to antibody levels, including a steady decline which could lead to seronegativity of some antibodies, cannot be confidently related to infection levels. E.g., untreated monkey IP67 had an initial antibody response which quickly declined and then remained very low. Measuring this animal's antibody levels might suggest that its immune system had cleared the infection. However, xenodiagnosis using PCR demonstrated the presence of both OspA and OspC sequences, showing that the animal remained infected.
9. Three of the untreated and one of the treated monkeys had high, sustained levels of multiple antibodies right through to the end of the experiment. These animal's immune systems had clearly mounted a vigorous defence, yet were unable to eradicate the infection, even when the most commonly recommended antibiotic was added and sustained beyond the usually recommended time limit.

The wealth of data and analysis provided by Embers et al makes a great contribution to the pool of knowledge, as well as raising important questions that must be addressed about Lyme disease.

One of those questions should probably be: why have those charged with protecting the health of the population of their respective countries not done either the same, or a similar experiment? Although the experiment was sophisticated and complex and surely represents a huge amount of difficult work, this is not beyond the scope of scientists in many countries.

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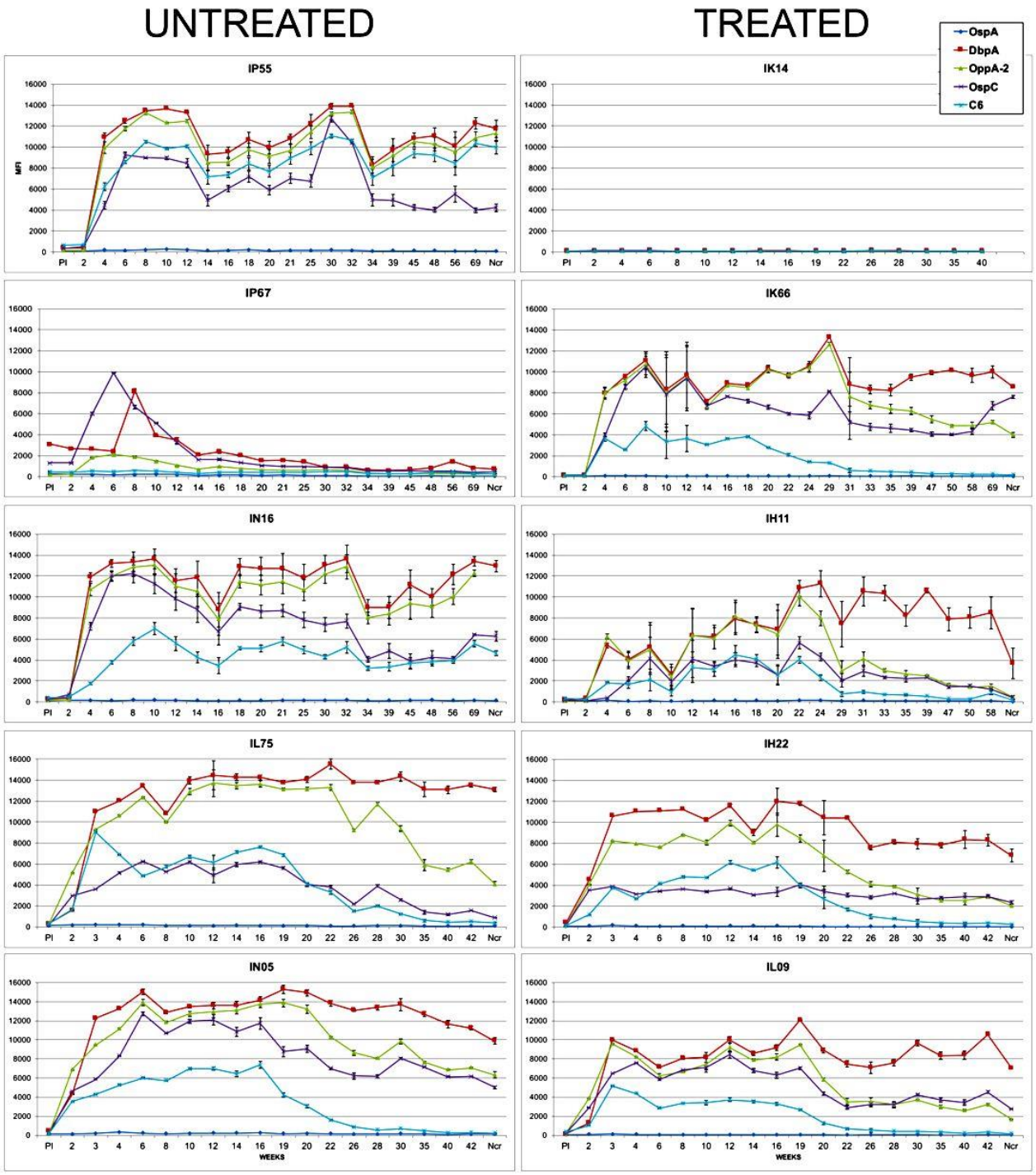

Figure 3, the original can be seen at: http://journals.plos.org/plosone/article/figure/image?download&size=original&id=info:doi/10.1371/journal.pone.0189071.g003