Statement from Peter Kemp MA about LDA's Comments on Microscopy in LDUK/LDA Q&A

http://www.counsellingme.com

Introduction

Darkfield microscopy is a technique sometimes described as 'light staining'. This is because it does not require any chemical staining to make minute details visible. It uses oblique illumination (illuminates the subject from the side – rather than from directly beneath). With this method the excess light which obliterates the tiniest subjects with bright-field microscopy, does not enter the microscope. Correctly illuminated subjects become a 'light source' (light stained). Therefore darkfield microscopy can detect subjects far beyond the theoretical limits of optical light microscopy.

Oblique illumination for microscopy dates back to the mid 1800s. Donne described spirochaetes in syphilitic samples in 1837. Darkfield microscopes have been used for observing spirochaetes in tissues including blood for over 100 years. In 1905 Shaudinn and Hoffmann identified Treponema Pallidum which causes syphilis and is a spirochaete similar in size to Lyme borreliosis species. In 1907 Jacquet and Sézary described the diverse morphology of this spirochaete including straight, non-coiled forms, 'chain of beads' and coccoid morphology. These morphology are also recognised forms of Lyme borrelia spirochaetes. In 1904, Ross and Milne described the presence of spirochaetes (now known as borrelia Duttoni) in African Relapsing Fever. By the mid 1900s numerous infectious spirochaetes had been discovered and described in humans and animals.

See:
William Cecil Bosanquet. 1911. Spirochaetes. A Review of Recent Work.
Robert E. Campbell , Paul , D. Rosahn. 1950. The Morphology And Staining Characteristics Of The Treponema Pallidum. Review Of The Literature

From: Questions posed by LDUK Lyme patients to LDA trustees

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> "4) What is the opinion of LDA on dark field microscopy? Does it have a place in Lyme diagnosis and if not, why not? Why is it used by many doctors abroad? Are they misguided?"
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> "Darkfield microscopy has been used in syphilis, but this was used on skin lesions, with more chance of viewing any spirochaetes, and not blood. In later infection particularly, Bb are only found in the blood in small numbers as they have worked their way into body tissues, so it is fairly unlikely that any will be present in a small blood smear. There is evidence that certain specialist microscopy techniques e.g. Focus Floating Microscopy, may be useful in examining tissue samples."

1/ The LDA state: "Darkfield microscopy has been used in syphilis, but this was used on skin lesions, with more chance of viewing any spirochaetes, and not blood."

It is true that darkfield microscopy is used in the diagnosis of syphilis. Even today, microscopists can enrol in courses dedicated to this routine diagnostic method. However, LDA state that "this was used on skin lesions… and not blood". This statement is misleading.

Firstly, syphilis in humans is often a sexually transmitted infection. Transplacental transmission does also occur with tragic consequences. In adults the infection is transmitted from person to person through direct contact with a chancre (syphilitic skin sore). Therefore the infectious spirochaete has no requirement to establish infection within a host's blood because it does not depend upon a blood sucking vector for transmission.

Secondly, syphilis spirochaetes enter and exit the blood and lymphatic systems for dissemination throughout the body. In established ('late' or 'tertiary') infection syphilis spirochaetes can be present in any organ of the body. They can be present in the liver, testes, brain etc., etc. Because these tissues are not tested, does not mean that syphilis spirochaetes are not present or would not be detectable.

Lyme Borreliosis spirochaetes are considered to mostly depend upon a blood-sucking vector for transmission. As with other infections that include a blood-sucking vector in their life-cycle they must infect the blood or die-out with the host. E.g., malaria, babesiosis, tick-borne relapsing fever, louse-borne relapsing fever, etc.

Furthermore, laboratories and scientists worldwide routinely use darkfield microscopy or variations of this method in the study of borrelia spirochaetes in culture, in ticks and in human tissues.

2/ The LDA state: "In later infection particularly, Bb are only found in the blood in small numbers as they have worked their way into body tissues, so it is fairly unlikely that any will be present in a small blood smear."

The unspecific term 'later infection' is unhelpful. 'Late infection' is a defined phase of syphilis infection. With Lyme borreliosis 'later infection' could mean anything. As LDA must know what they mean by 'later infection' because they describe a specific infection process associated with that stage – will they kindly inform us what time period this encompasses and who defined it?

The term 'small blood smear' is not clear either, though presumably refers to the commonest type of blood sample used in histology – a 'thin film smear'. A thin film blood smear is usually around 5 to 7 microlitres which allows cell-counting and observation of abnormal cells. However, in thin-film smears only a proportion is generally examined by microscopy, e.g., a 4cm x 2cm thin-film smear is 8 million uM^2 (square microns). A 100um diameter field of view is ~800 uM^2. So 200 fields

of view (e.g. as used for malaria diagnosis) is only 1/50th of the sample or 1/10th of a microlitre.

If LDA will provide an explanation of what 'small numbers' means, I will complete the above calculations to determine the likelihood of an automated scanning system detecting a spirochaete according to LDA's described methods.

These figures aside, why have LDA chosen to limit their remarks to a 'blood smear'? A thin film smear of fresh blood is not routinely used by anyone for observation of Lyme borreliosis spirochaetes (Mattman and others did use smears but these were then ***cultured***). Darkfield microscopy can reliably locate spirochaetes when present in a fresh wet-drop preparation of peripheral blood. More often, a culture period is required to allow the spirochaetes to grow and/or emerge from cells. Thin film smears of fresh blood are irrelevant when compared to vastly superior sample types for studying spirochaetes.

Blood is a body tissue. It is in fact defined as 'connective tissue'. This is not medical science, it is simple biology.

A 'successful' borreliosis infection must spread to the blood in order to infect a new blood-sucking vector and thence transfer to the next mammal host. If it does not infect the blood then it cannot perpetuate – it will die out with the host.

In 2010, Tappe *et al* studied *Ixodes Ricinus* ticks in Hanover, northern Germany (http://www.parasitesandvectors.com/content/7/1/441). They found 30% of adult male ticks were positive for borrelia burgdorferi s.l. spirochaetes. Adult male ticks have only fed twice, at the larval and nymphal stages. Occasionally ticks take meals from multiple hosts (following incomplete feeding) and transovarial transmission (they hatched from eggs, already infected) can account for a percentage of infected adult ticks.

Strube et al stated in 2010: "In Germany, approximately 1% of larvae, 4 to 18% of nymphs and 10 to 35% of adult ticks are infected with B. burgdorferi sl". (http://www.parasitesandvectors.com/content/3/1/69).

A considerable proportion of the adult male ticks in these studies acquired borrelia infection having fed only twice. In the case of males, the total volume of blood ingested with both meals is a few microlitres, yet this was sufficient to infect the tick. They did not get the infection by sucking out the host's brains or gorging on its liver. They got it from the host's blood.

3/ The LDA state: "There is evidence that certain specialist microscopy techniques e.g. Focus Floating Microscopy, may be useful in examining tissue samples."

This statement is a triumph of spin. The implication is that LDA are not simply aware of the existence of 'Focus Floating Microsopy' (FFM), but that they understand it and recognise it as a 'specialist' technique. Neither of these are true.

The 'Focus Floating' part of FFM, refers to the fact that instead of scanning a microscope slide in 2 dimensions, X and Y, the microscope is programmed to also alter the focus in the Z plane up to 4 microns. For automated systems this is a useful modification. To microscopists that work manually with wet-drop/live tissue preparations it is completely meaningless. It is impossible to achieve a wet-drop sample with a depth less than ~10 microns. So every microscopist studying spirochaetes in live tissue or live cultured spirochaetes must continuously adjust the focus of their instrument. It would be nice to have a machine to do it for you, but that is what the fine focus knob is for.

Other than the above, FFM is an immunohystochemical method which nearly everybody studying Lyme and borrelia does at some point. It is just antibody staining; often with 'off the shelf' products, of target proteins with some kind of optical marker which is usually fluorescent.

There is nothing whatsoever 'specialist' about Focus Floating Microscopy. There is nothing which enables it to detect spirochaetes that cannot be detected by normal darkfield and immuno-fluorescence methods.

Conclusion

LDA's answer to patient's questions about microscopy are ill-informed and misleading. Whether this is misinformation or disinformation I will leave to the reader.