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Routine diagnostic methods for sensitive detection of louse-borne relapsing fever due to Borrelia recurrentis based on PCR and fluorescent microscopy used in case cluster in southern Germany 2015

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Background: The human body louse transmits *Borrelia recurrentis,* the causative agent of louse-borne relapsing fever. In the past centuries, large outbreaks have been described claiming many lives during hard times (“lousy times”) such as famines and wars. The untreated lethality if the disease can be above 20%; and up to 80% of treated patients develop Jarisch-Herxheimer reactions, which often require hospitalization and can be fatal.

Material/methods: In summer to autumn 2015, more than 20 cases of louse-borne relapsing fever have been diagnosed in southern Germany. All patients were young male asylum seekers from east Africa who arrived via the Italian route in southern Germany. Several patients had to be treated in intensive care wards, one patient died after initiation of treatment. The main symptoms were fatigue and fever, both quite common symptoms with vast differential diagnoses. As due to the large numbers of asylum seekers, they are housed in many different locations within Germany, many facilities lack proper access to experienced diagnostic facilities. Therefore, fast and sensitive diagnostic tools are needed to allow detection of infected subjects.

Results: Diagnosis is regularly made by microscopy of stained blood films; however, this greatly depends on the availability of experienced microsopists to detect also low concentrations of spirochetes in the sample. So within the outbreak, we have evaluated a sensitive PCR method using extracted DNA from 500µl of EDTA blood and modified 16s primer sequences to detect more sensitive sequences form *B. recurrentis* which are not easily picked up by regular 16s primers in low concentrations. We also evaluated a DAPI staining protocol to allow for reading of blood films and thick smears using fluorescent microscopy, thus increasing the sensitivity of the investigation also for

less experienced microsopists. *B. recurrentis* spirochetes can thus easily be identified and differentiated from artefacts within the sample.

Conclusions: Using tailored 16S-primers and fluorescent-microscopy, the diagnosis of *B. recurrentis* in EDTA blood is fast and reliable also in submicroscopic densities. Fluorescent staining with DAPI facilitates the detection of low-level bacteremia and reduces artefacts(Fig1).

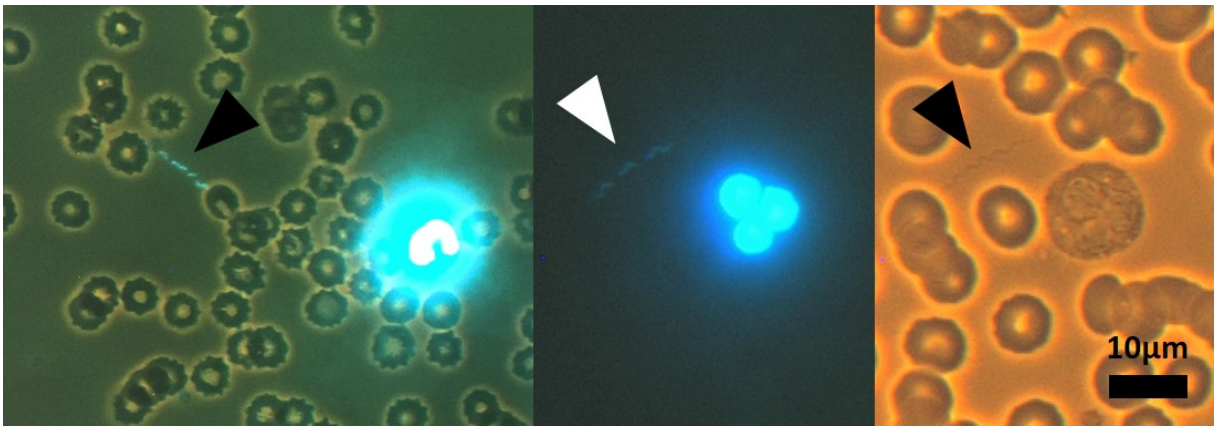

Fig 1.