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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 lethality of untreated disease can be above 20%[1]. Unfortunately, also up to 80% of treated patients develop Jarisch-Herxheimer reactions, which often require hospitalization and can be fatal [2]. Rapid diagnosis is therefore desirable. Due to the very limited number of cases in regions with good laboratory infrastructure, little is known about the sensitivity and specificity of current microbiolgical diagnostic procedures for louse-borne relapsing fever.

Overview: Beginning in late summer 2015, the first cases of louse-borne relapsing fever were diagnosed in Southern Germany [3,4]. Till March 2016, a total of 42 cases have been diagnosed. 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 [3]. 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: A total of 42 cases of Louse-Borne relapsing fever were diagnosed. Suspected cases were identified primarily by microscopy of stained blood films as well as clinical presentation.
Out of the 42 cases, 37 blood samples were examined at the reference center for borrelia or the Max von Pettenkofer-Institute respectively (Table 1).

Procedure:	**Positivity:**
Positive by microscopy (Giemsa)	**≥ 80%** [34/42]
Modified 16s PCR	**100%** [37/37]

Table 1: Positivity rate of the samples by method.

For all samples a modified 16s PCR from DNA extracted from 500µl of EDTA blood was performed. The primers and protocol are shown in Table 2. The PCR showed excellent sensitivity also in samples shipped for several days at ambient temperature.

Recurr. FOR: 5‘ ggc tta gaa cta acg ctg gca gtg c 3‘			
Recurr. REV: 5‘ ccc ttt acg ccc aat aat ccc ga 3‘			
Protocol:			
94° C	5min	initial heating phase	
94° C	15s	denaturation	40x
54° C	30s	annealing	
70° C	1min	elongation	
72° C	7min	terminal elongation	

Table 2: Modified 16s PCR for *B. recurrentis*. [5]

As 16s sequences are highly homologous in borrelia, a differentiation requires the sequencing of further genes such as *glpQ uvrA* and *fla*.

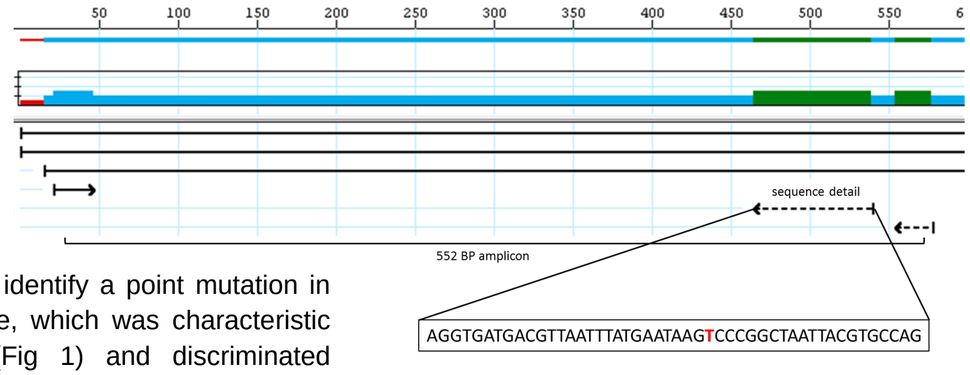

Figure 1: PCR reaction schematic with highlighted sequence.

We were able to identify a point mutation in the 16s sequence, which was characteristic in all isolates (Fig 1) and discriminated against the otherwise identical sequences of *B. duttonii*, *B. crocidurae*, *B. miyamotoi* and other borrelia in GenBank.

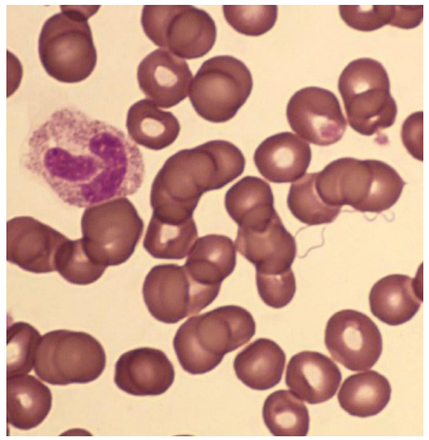

Figure 2: Giemsa stained blood film with *B. recurrentis*.

Out of the total 42 cases, 34 had confirmed positive blood smears in microscopy (Fig 2). A total of eight samples were negative or unclear. In our hands, a DAPI staining protocol using DAPI containing mounting medium was used after Giemsa staining or after Methanol fixation. UV-fluorescence microscopy showed vastly simplified detection and less artefacts (Fig 3).

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

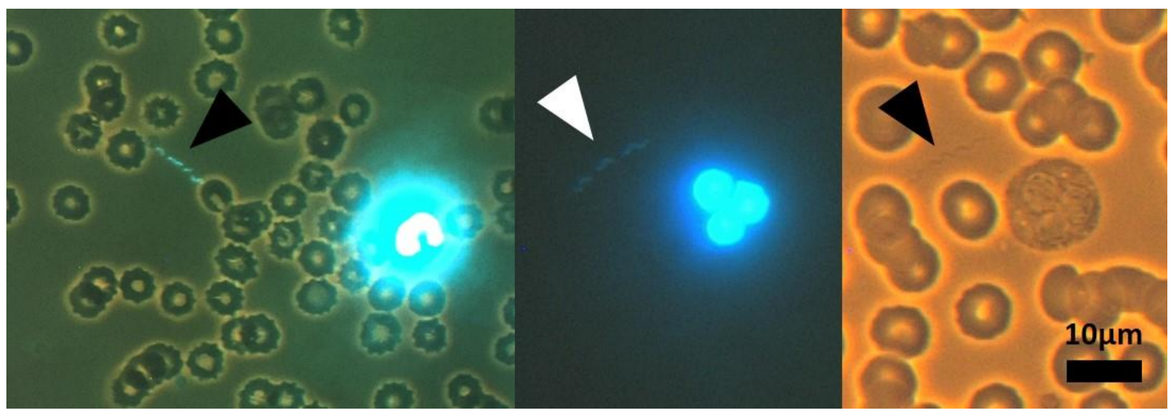

Figure 3: Bright field microscopy as well as DAPI fluorescence microscopy of a *B. recurrentis* blood film.

1: Bryceson et al. 1970
2: Butler et al. 1978
3: Hoch et al. 2015
4: Wieser et al. 2016 (DMW accepted)

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