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Diagnosis of human trypanosomiasis, due to *Trypanosoma brucei gambiense* in central Africa, by the polymerase chain reaction

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Abstract

During a mass screening of sleeping sickness conducted in 1998 and 1999, and involving 27 932 persons in Cameroon and the Central African Republic, we tested the polymerase chain reaction (PCR) on whole blood for the diagnosis of human African trypanosomiasis due to *Trypanosoma brucei gambiense*. The 1858 samples obtained were from 4 groups: 155 infected patients, 1432 serological suspects detected by the card agglutination test for trypanosomiasis (CATT), 222 negative controls living in the prospected area (negative with the CATT and parasitological methods), and 49 negative controls (CATT and parasitological methods) and unexposed to the disease (Europeans). The technique of DNA extraction used made it

gical suspects that were negative according to the other techniques used were cultured with the kit for in-vitro isolation of trypanosomes in the field (KIVI; AERTS *et al.*, 1992).

The blood samples obtained in vacutainers containing EDTA were stored at about 4°C and used as templates for PCR after DNA extraction with the kit Ready AMP™ genomic purification system (Promega, Madison, WI, USA) at the Molecular Biology Laboratory of CEAC. This kit allows DNA extraction from more than 100 samples in a single day. The technique initially described by PENCHENIER *et al.* (1999) was modified by increasing the duration of the incubation period at 56°C to 30 min (instead of 20 min) and of incubation at 100°C to 15 min (instead of 10 min). The PCR technique was performed by using TBR1 and TBR2 *T. brucei s.l.* specific primers (MOSER *et al.*, 1989).

Study groups

The survey included 27 932 persons: 13 092 from Fontem, 5255 from Campo, 6696 from Bipindi, and 2889 from Batangafo. With the 49 Europeans used as negative controls, a total of 27 981 subjects were enrolled in this study.

We carried out PCR on 1858 blood samples corresponding as shown to the 4 following groups.

- Patients: 155 CATT positive and positive with the QBC and/or lymph-node aspiration.
- Serological suspects: 1432 CATT positive, but parasitologically negative.

strongly serologically suspect but PCR negative. Finally we followed-up the exposed negative control individuals who were PCR positive during the first sampling.

Results

First survey

The results obtained from mass screening are shown in the Table.

Of the 155 patients, only 1 was PCR negative. These patients was detected by CATT and diagnosed by lymph-node puncture. Among the serological suspects, 50 were PCR positive and 1382 PCR negative, which yields a positivity rate of 3·5%. Among the exposed controls, 3 were PCR positive. In the group of non-exposed controls, none was positive.

Reproducibility of the results

Of the 22 PCR positive patients re-examined 1–3 months after diagnosis, and before treatment, all remained positive by PCR; 33 PCR negative persons followed during 6 months remained negative.

To study the influence of blood conservation, we carried out PCR on samples stored during 1 month at room temperature in the laboratory and others stored for >3 months at 4°C. The results were identical to those obtained on samples treated immediately after sampling.

Survey of serological suspects and negative control individuals

The results obtained for control individuals are shown in the Table.

control or 49 unexposed control individuals. PCR was negative for 96·9% (1383/1427) of the serological suspects. The increase in detected patients, among the re-examined suspects, was 12·8% (5/39).

Discussion and Conclusion

Our results show that PCR used according to our technique, compared to those used by KANMOGNE *et al.* (1996) or KABIRI *et al.* (1999), is more sensitive.

The infection in 1 PCR negative patient was detected by lymph-node puncture and confirmed by KIVI, meaning that trypanosomes were present in the blood and PCR should have been positive. Repeated PCR amplifications were carried out on this patient's blood samples from 1 to 4 months after treatment, but the results were all negative. However, a registration error cannot be excluded. The discordance noted between the PCR and parasitological methods is possibly due to poor or erroneous labelling of the sample tube during the survey. It is thus possible that this patient was PCR positive.

Despite this single exception, PCR remains a very sensitive technique since, after controls, 99·4% (159/160) of the patients were positive. Our PCR protocol is also very specific. On the one hand, the 111 PCR negative serological suspects were found to be uninfected during follow-up. On the other hand, all the non-exposed negative controls and 98·6% (219/222) of the exposed negative controls were PCR negative. For the 3 exposed negative control individuals (from the group of 222 of these cases. This system is more flexible and cheaper than sending a medical team to find all the serological suspects. There remains the problem of the PCR positive serological suspects lost to follow-up, the proportion of which was, in this study, around 9% (4/43). But how many patients would we have lost if we had had to seek 1432 serological suspects?

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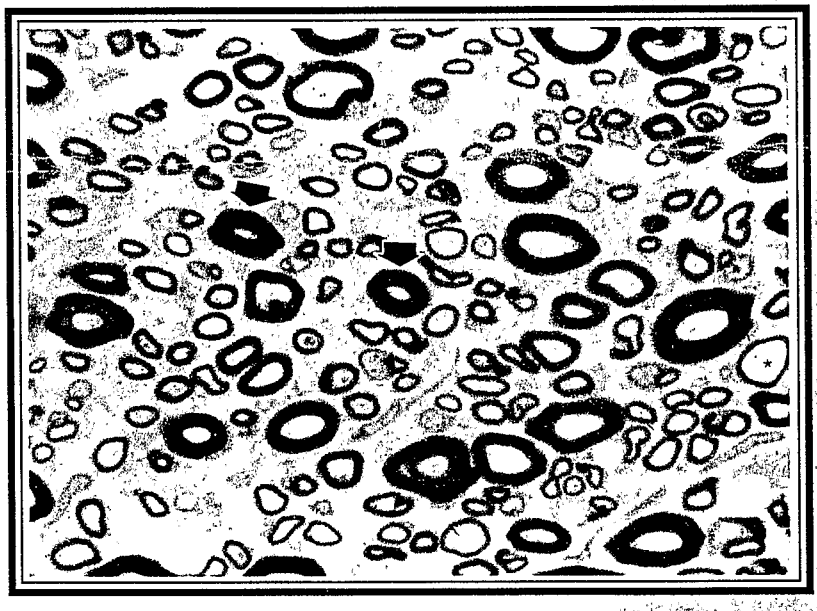

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