Short communication

A new method for identifying blood meals of human origin in tsetse flies

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Abstract

A new sensitive technique using the electrophoresis of superoxide dismutase to distinguish between tsetse blood meals of human and non-human origin is described. In Côte d’Ivoire, 602 blood meals were collected; 170 were from man (28.3%), 377 from animals (62.6%) and 55 were unidentified (9.1%) because no pattern was observed. When calculating the index of epidemiological risk, it is strongly correlated with the incidence of sleeping sickness cases. © 1997 Elsevier Science B.V. All rights reserved

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1. Introduction

The calculation for a given area of the index of epidemiological risk should lead to major improvements in human African trypanosomiasis control (Laveissière et al., 1994). An important component in such an estimate is the number of blood meals taken from man by the tsetse fly vector, which may, for instance, increase at the beginning of a period of high transmission. There may be no need to identify

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the source of every blood meal, as is normally done and the widely used comple-
ment fixation test may fail to identify more than 20% of blood meals collected
(Staak et al., 1986). Instead the proportion of human to animal feeds would suffice
for many purposes, particularly if changes in feeding patterns are suspected. If valid
results are to be obtained, samples must be protected against deterioration, which
may be rapid in tropical conditions.

This paper presents a sensitive technique using the electrophoresis of superoxide
dismutase to distinguish between tsetse blood meals of human and non-human
origin.

2. Materials and methods

2.1. Collection of midguts

After dissecting a trapped tsetse fly in saline on a microscope slide, the whole
midgut was removed and placed at a marked position on a Whatman No. 1 filter
paper. The samples were stored in a glass vial, containing silica gel, and kept
refrigerated until required.

2.2. Sample preparation and isoenzyme electrophoresis

The piece of filter paper carrying the squashed midgut from each fly was cut out
and place into 30 \( \mu l \) of enzyme stabilizer (Miles and Ward, 1978), in a well of a
microtitre plate kept on ice. After 20 min, the extraction product was re-suspended
thoroughly by repeated gentle pipetting. Five applications in one position totalling
2 \( \mu l \) from each extract were then placed at the origin of a cellulose acetate plate,
ready for electrophoresis. Ten samples were placed on each plate, together with
control extracts from human blood and from the midgut of a starved fly. Elec-
rophoresis, by the Helena system, and staining for superoxide dismutase (SOD; EC
1.15.1.1) are described by Truc and Tibayrenc (1993).

3. Results and discussion

In man, two autosomal loci SODa and SODb determine respectively the soluble
and mitochondrial forms of the enzyme (Creagan et al., 1973); at pH 7.0 the SODa
isoenzymes migrate anodally and the SODb cathodally. SODa is found in all tissues
except polymorphonuclear leucocytes (Beckman et al., 1973). A preliminary study
showed that SOD from human red blood cells had a different electrophoretic
mobility than SOD from tsetse midgut. Furthermore, no SODa variability was
observed among different human blood samples nor among several midguts from
wild flies. When comparing human blood with blood of other mammals (cattle, pig,
sheep, horse and mouse), the patterns observed for human blood was clearly
different from other bloods and also from the midguts of starved tsetse flies.
Although tsetse can feed on other species of animals, previous studies show that 90% of meals are taken either from man, pig or dog by *Glossina palpalis* in Côte d'Ivoire (Staak et al., 1986). Thus, our technique could distinguish human from non-human blood in a tsetse midgut.

After staining, the patterns appeared as white zones on an orange background. Interpretation must be done immediately because sometimes an indistinct pattern, presumably as a result of a small amount of enzyme, disappeared when fixed with acetic acid. The patterns observed clearly showed those from man had a significantly faster mobility of SOD than any non-human blood meal.

In Côte d'Ivoire, 602 blood meals were collected from *G. p. palpalis* in the Sinfra area; 170 were found to be from man (28.3%), 377 from animals (62.6%) and 55 were unidentified (9.1%) because no pattern was observed. These unidentified cases are probably due to digestion of a blood meal, so that SOD had been wholly degraded.

When calculating the index of epidemiological risk (Laveissière et al., 1994) from those results, it is strongly correlated with the incidence of sleeping sickness cases (Laveissière, unpublished results). A large number of human blood meals in an area indicates a high risk of disease transmission.

Although this technique does not allow species identification but only distinguishing human from non-human feeds, the number of unidentified blood meals seems lower than using the technique of Staak et al. (1981); 9.1% instead of 23.3%. Work is in progress in order to reduce the cost of the new electrophoretic approach. Further comparative trials should be carried out, especially with the ELISA technique used for rapid detection of human blood meals in mosquitoes (Savage et al., 1991).

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**References**


