Polymerase chain reaction as a diagnosis tool for detecting trypanosomes in naturally infected cattle in Burkina Faso

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Abstract

African animal trypanosomoses constitute the most important vector-borne cattle diseases in sub-Saharan Africa. Generally it is considered that there is a great lack of accurate tools for the diagnosis of the disease. During a trypanosomosis survey in the agro-pastoral zone of Sideradougou, Burkina Faso, 1036 cattle were examined for trypanosomes using microscopy. The PCR was applied on a subset of 260 buffy-coat samples using primers specific for \textit{Trypanosoma congolense} savannah and riverine-forest groups, \textit{T. vivax}, and \textit{T. brucei}. Parasitological examination and the molecular technique were compared, showing a better efficiency of the latter. In the near future, the PCR is likely to become an efficient tool to estimate the prevalence of African trypanosomoses in affected areas. ©1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

African animal trypanosomoses are considered as the most important cattle disease transmitted by vectors in sub-Saharan Africa (FAO, 1994). Non polluting control methods are
currently used and can prove successful under certain conditions (Bauer et al., 1995). However, these methods require a thorough knowledge of the epidemiological situation, which is time and labour consuming. Until now, there has been no reliable diagnosis test able to assess the true prevalence of trypanosomoses in cattle: the classical microscopical examination, either by the Woo technique (Woo, 1970) or by the buffy-coat method (Murray et al., 1977), lacks sensitivity (1000 tryps/ml). Antibody-detection ELISA has the advantage of being economic and is applicable at a large scale, but does not differentiate between past and present infections, nor distinguish between species of trypanosomes. Moreover the test is not suitable for the diagnosis of an individual animal. Antigen-detection ELISAs have been shown to lack specificity and also sensitivity (Desquesnes, 1996). The polymerase chain reaction (PCR), using primers designed from satellite genomic DNA sequences specific of different taxonomic groups, has proved the most sensitive and specific experimental technique to detect trypanosomal DNA in either the vector or the host (Moser et al., 1989; Masiga et al., 1992; Majiwa et al., 1994; Solano et al., 1995; Reifenberg et al., 1997; Lefrançois et al., 1998). However, PCR has been scarcely applied to assess the prevalence of trypanosomosis on field samples, due to its reputed time-consuming and prohibitive cost aspects, and the requirement for technical expertise.

In the agro-pastoral zone of Sideradougou (Southwestern Burkina Faso), a multidisciplinary study has been undertaken, the aim of which was to identify the key factors affecting the presence of tsetse flies. All data on entomology, parasitology, livestock, hydrographic network, environment, land use, have been geographically referenced and included in a Geographic Information System, in order to deduce indicators of areas at higher risk of transmission (de La Rocque et al., 1998). Tsetse had been completely eradicated from the area some years ago by the combined use of impregnated screens and sterile insect technique (Politzar and Cuisance, 1984). After the program had been stopped, tsetse reinvaded the area and are now occupying the galleries all along the hydrographic network in the area (de La Rocque et al., 1998). A total of 70,000 cattle were estimated to occupy the area in 1988 and a new estimation is in preparation (Michel et al., 1999). A rapid and reproducible evaluation of the prevalence of trypanosomosis is required together with other key indicators, in order to decide on appropriate strategic interventions for a sustainable control of the disease.

The objective of the study was to apply the PCR technique on a field survey for the first time in West Africa in order to assess the prevalence of trypanosome infections in cattle, with a minimum possible cost and maximum speed, and to compare the results obtained on a random sample with those using a parasitological method.

2. Material and methods

2.1. Study area

The survey was made in November 1997 in the northern part of the agro-pastoral zone of Sideradougou (see Fig. 1). An exhaustive and georeferenced survey of the geographical distribution of cattle had been made in this northern part which lead to a count of 16,600
Fig. 1. Map of the Sideradougou area, Burkina Faso, and geographic location of the sampled herds.
heads of cattle (Michel et al., 1999). The Sideradougou area harbours a typical southern Sudanian vegetation with riverine forests along the hydrographic network. The zone is crossed by two majors rivers, the Koba and the Tolé, and a secondary hydrographic network appears during the rainy season (June to October). Riverine tsetse flies of the *palpalis* group (*Glossina tachinoides* and *G. palpalis gambiensis*) constitute the potential vectors of trypanosomes in the area.

### 2.2. Epidemiological survey and sampling methods

A representative stratified random sample (e.g. Cochran, 1963) of 1036 cattle was selected on the basis of the above mentioned exhaustive inventory (see Fig. 1), to allow an estimate of the prevalence in the total sample with a maximum error of 6%.

From each animal, 5 ml of blood were collected from the jugular vein in a heparinised tube, and brought back to the field camp which was in the immediate vicinity. Two capillary tubes were prepared from each blood sample. The first tube was used to measure haematocrit and to detect trypanosomes by examination of the buffy-coat using dark-ground microscopy (Murray et al., 1977). The second tube was broken just under the limit between the buffy-coat and the red cells, and the buffy-coat was mixed with 30 µl of sterile distilled water in a 0.5 ml eppendorf tube. After agitation, samples were frozen before further analysis.

Out of the total population of cattle examined, a randomly drawn subset of 260 buffy-coat samples was analysed for detection of trypanosomal DNA by PCR.

Trypanosome prevalences were calculated with normal confidence intervals.

### 2.3. DNA amplifications

The 0.5 ml eppendorf tubes containing the buffy-coat samples in sterile distilled water were brought back to CIRDES where all subsequent technical procedures were done. First, 100 µl of 1% aqueous solution of Chelex® (BIORAD) were mixed with the buffy-coat of each sample and the solution was incubated for 1 h at 56°C and 30 min at 95°C (Walsh et al., 1991). Thereafter each tube was centrifuged for 2 min at 15 000 g and stored at −20°C.

Primer sets specific for *Trypanozoon*, the savannah and riverine forest types of *T. congolense*, and *T. vivax* were used (see Table 1). All these primer sets have been evaluated for sensitivity and specificity by the authors mentioned as reference, and also by others (see for example Solano et al., 1995). Standard PCR amplifications and electrophoresis were carried out as previously described (Solano et al., 1995) except that standard PCR mixes were done simultaneously for 50 samples and included two negative controls: the first was
placed in 25th position, and the second at the end. A positive control with reference DNA of each trypanosome type was also included in each PCR assay. Amplification products were resolved in 1.6% agarose gels (14 cm), stained with ethidium bromide. When the amplification reaction gave a signal of the expected size according to the set of primers used without any signal in the negative controls, the infection was considered to be characterised.

3. Results

3.1. Parasitological results

The parasitological prevalence was 5.3(±1.3)% in the total sample of 1036 individuals (55 positive cases). On the 260 selected individuals, it was 4.2(±2.4)% (11 positive cases). These two numbers were not statistically different.

Out of these 260 animals, most of the infections found by microscopical examination were attributed to *T. congolense* (7 cases), followed by *T. vivax* (3 cases) and *T. brucei* (1 case).

3.2. PCR results

The overall prevalence obtained on the subset reached 11.5(±3.9)%. Out of the 30 animals found infected by PCR, *T. congolense* riverine-forest type was never observed. *T. congolense* savannah type was more frequently detected than *T. vivax* and *T. brucei* (see Fig. 2), as it was found in approximately 80% of the infected animals. Four individuals had an infection with two different trypanosomes (*2 T. congolense* savannah/*T. vivax* and *2 T. congolense* savannah/*T. brucei*).

3.3. Comparison between parasitological and PCR results

The $\chi^2$ test on the original data set showed a significant difference between the prevalence using PCR and the parasitological one ($p < 0.01$). Indeed it represented more than two times the prevalence detected parasitologically (11.5% vs. 4.2%). The main difference consisted in the number of positive cases of *T. congolense* savannah and *T. brucei* (see Fig. 3), which dramatically increased using the molecular technique.
Fig. 3. Number of positive infections detected by PCR and by microscopical examination. Note: the number of infections is higher than the number of cases because of the four cases of mixed infections detected by PCR.

Out of the 11 animals which had been found infected by microscopical examination, PCR gave interpretable signals on 10, which represents a 91% identification. On the other hand, the PCR gave clear amplification signals on 20 animals not detected positive by parasitological examination. This highlights the difference between the two techniques because of the 30 cases positive by PCR, two-thirds (most of which had a haematocrit value below 25%) were negative using the parasitological technique. Furthermore, PCR allowed the accurate diagnosis of four mixed infections, which could not be detected by parasitological examination.
At no time during the study, the negative controls showed a visible signal. Moreover, all the primer sets used had been carefully checked for absence of cross-amplifications (see also Majiwa et al., 1994; Solano et al., 1995).

4. Discussion

The PCR technique using primers designed on repetitive genomic DNA sequences of trypanosomes had already been used for epidemiological purposes. However, such investigations focused essentially on the identification of trypanosomes in tsetse flies (McNamara et al., 1995; Masiga et al., 1996; Solano et al., 1996; Reifenberg et al., 1997), and to a much lesser extent in cattle. The technique has been verified on blood samples of experimentally infected animals, confirming its higher sensitivity and specificity when compared to parasitological techniques (Moser et al., 1989; Clausen et al., 1998). In the present work, we used the PCR technology as a diagnosis tool to assess the prevalence of animal trypanosomosis on samples collected in the field, in West Africa, at the geographic scale of an agro-pastoral zone.

Using the PCR, a significantly higher prevalence was obtained than by classical parasitological examination. This was due to the higher sensitivity of the molecular technique which is able to detect less than one trypanosome per ml under experimental conditions (Moser et al., 1989). Such prevalence differences as obtained by the two techniques would have a significant impact on the strategy selected for the control of the disease in affected areas. Interestingly, we demonstrated that analysing a random sample of cattle, the PCR technique could be applied in a rapid and simple way (three weeks of work for one technician to analyse the 1040 PCR samples: 260 individuals * 4 primer sets). We showed, as others did previously (Penchenier et al., 1996; Katakura et al., 1997), that no labour and time-consuming DNA extractions using classical protocols were anymore needed for epidemiological surveys. By using chelating resins, the available DNA in the samples could be properly amplified. From a technical point of view, we believe that PCR applied on buffy-coat samples seems more promising than on whole blood extracted from filter paper, as the concentration of trypanosomes is higher in the buffy-coat (Woo, 1970; Murray et al., 1977). Moreover the technique here appeared quite economic (0.5 US$ per head of cattle) when compared to previous studies (for review, see Desquesnes and Tresse, 1996). Progress in lowering the costs of reagents used and minimising time spent on the PCR (Mai et al., 1998), will facilitate the diagnosis of field samples.

One animal presented an infection (diagnosed by microscopy as *T. congolense*) that was not recognised by the sets of primers used. This could reflect either a technical problem, or the presence of trypanosomes which could not be recognised by the primers used, as has been reported previously (Katakura et al., 1997). Nevertheless, the technique used here appeared satisfactory. It should also be noticed that in the present survey, 12.4% of the 1036 cattle had a haematocrit value below 25% (such a value is a sign of anaemia): this percentage is consistent with the trypanosome prevalence found by PCR. Among the individuals showing such a low haematocrit value, up to 40% were found infected with trypanosomes using PCR (data not shown). These results confirm findings in this study area reported elsewhere (Lefrançois et al., 1998) and underline
that the haematocrit value is a good indicator of trypanosomosis in cattle (Féron et al., 1987).

5. Conclusion

Taking into account current views on sustainable control of African trypanosomases (Hendricx et al., 1997), a need arises for tools that can assist in a rapid appraisal of the parasitological situation in agricultural and livestock areas: the use of the PCR to assess trypanosome prevalence in cattle seems a valuable tool for this purpose.

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