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Standardized Approaches to Assess Vector Competence

Chandini Sharma*

Department of Plant science, University of Calicut, Kerala, India

***Corresponding author:** Chandini Sharma, Department of Plant science, University of Calicut, Kerala, India; E-mail: chandini1992@gmail.com

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Introduction

The development of good treatment and control strategies to protect livestock against trypanosomiasis requires accurate data regarding the disease epidemiology. This in turn depends on accurate diagnosis and definitive identification of causative trypanosome species. Most epidemiological studies have relied on parasitological methods for the demonstration of trypanosomes despite their limitations in terms of sensitivity and practicability. Serological tests such as AB-ELISA detection methods are not reliable for differenting current or post treatment infections. Ag-ELISA assays have also been shown to be of insufficient sensitivity for any diagnostic value. Accurate detection of trypanosomes in both the host blood and vectors now heavily depends on the highly sensitive and specific Polymerase Chain Reaction (PCR). Species specific primers amplifying pathogenic trypanosomes have been designed and used to characterise trypanosomes and in epidemiological studies. However, pathogenic trypanosomes are known to occur and overlap in most of the tsetse infested belt.

Trypanosomiasis Control Strategies in Cattle

The diagnostic performance variables such as sensitivity and specificity for each of the assays used in this study have been estimated in separate studies describing them. However besides estimating diagnostic performance variables of available PCR tests, assessing the tests for agreement is important for practical applications of the different PCR diagnostic assays. The choice of the method to use for each study should consider the cost implications as well as the need for accuracy. The two ITS based assays utilizing the same diagnostic target showed a higher diagnostic capacity compared to the species specific tests. The difference was mainly due to the high numbers of *Trypanosoma vivax* picked by the based assays. Accurate diagnosis of *T. vivax* species is important since it is the etiological cause of the severe hemorrhagic disease in cattle. This is made even more important from the fact that *T. vivax* is also transmitted mechanically. This means that whereas tsetse control would bring the prevalence other pathogenic trypanosomes down, *T. vivax* would possibly persist. Diagnosing *T. vivax* therefore, would spell the need for other trypanosomiasis control strategies in cattle such as chemotherapy besides tsetse control. Although the single PCR ITS based diagnostic test is reported to have a low sensitivity against *T. vivax*, our results indicated it was able to detect much more (17.5%) positives for *T. vivax*, compared to the TVW primers (3.9%). These results support other studies suggesting that TVW primers target certain DNA sequences that are not conserved in all *T. vivax* isolates, resulting to false-negatives. In addition, low sensitivity could be due to the TVW primers targeting molecules that are low in copy numbers as compared to ITS-PCR whose target gene could be higher in copy numbers.

Cost Constraints of Species-Specific Diagnostic Method

Trypanosomiasis in the field will present both in the chronic and acute forms depending on whether it is in the endemic or epidemic epidemiological form. Whereas parasitological methods are reported as having an almost equal sensitivity to PCR in detecting infections in the acute phase, they exhibit very low sensitivity in the chronic phase characterized by low parasitaemia, in which PCR will be two to three times more sensitive. There have been debates on the relative merits of microscopy and PCR methods for the detection of trypanosome infection but this should focus on advantages of each method for specific purposes. For instance, field studies comparing the use of PCR and microscopic observation of the buffy coat have reported higher sensitivity with PCR, which should be important in epidemiological study. The three tests did not differ significantly in detecting *T.brucei* and picked almost equal numbers of *T. brucei*. The TBR 1 and TBR 2 primers target a 177 bp repeat sequence which occurs in a high copy number of approximately 1000 copies, which explains the comparable sensitivity of these primers with the ITS based assays. A statistical analysis of the test agreements indicates a significant difference in the diagnostic capacity between the species-specific primers tests and the other two universal tests largely due to *T. vivax* numbers. Between the two universal tests, the McNemar's test for bias was not significant and the kappa value indicated reasonable agreement, implying that the two tests presumably detect the parasites equally well. Prohibitive costs and widespread perception that diagnostic PCR technology is complex slows down its adoption. For Instance in this study, while using the species-specific diagnostic method, five different PCR assays per sample were required to screen for *T. vivax, T. brucei* and the three subtypes of Congolese; savannah, kilifi and Tsavo. The tests consumed more time and labour, and a higher cost compared to the two ITS based tests. If the cost constraints are overcome, efforts should be directed towards making diagnostic PCR technology automated, minimizing sample handling and decreasing the possibility of contamination, while raising the potential to function efficiently in the hands of moderately trained technical staff. The use of ITS based primers as a universal diagnostic test for all pathogenic trypanosomes considerably overcomes the above constraints. By reducing the number of reactions per sample to one or two, the tests effectively reduces the cost of PCR by two to three times.

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