A survey of trypanosomosis in Zambian goats using haematocrit centrifuge technique and polymerase chain reaction

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ABSTRACT

The incidence of trypanosomosis was determined using the haematocrit centrifuge technique (HCT) as well as polymerase chain reaction (PCR) on 120 goat blood spots on filter paper. Both techniques failed to detect a positive reaction, implying that factors such as age, healthy appearance and small sample size notwithstanding, trypanosomosis does not seem to pose a serious threat to goat health in the districts from where the animals originated.

Key words: goats, PCR, trypanosomosis, Zambia.

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Goat-keeping is an important and an integral part of rural agriculture in the marginal agricultural areas of Zambia. According to the statistics available at the Livestock Economics and Epidemiology Unit, goats are widely distributed in the country but the pattern of distribution is skewed with over 60 % of the goat population concentrated in the Luangwa-Zambezi Rift Valley of the semi-arid region[16]. These areas are characterised by poor crop production, feed scarcity, and cattle do not thrive because of the semi-arid conditions. All the goats utilised in this study originated from Luangwa-Zambezi Rift Valley area that has been documented to be heavily infested with tsetse flies, especially *Glossina morsitans morsitan* and *Glossina morsitans centralis*[2]. It has been estimated that about 20 % of national cattle is at the risk from trypanosomosis. Survey activities undertaken by the Department of Animal Health and Production in collaboration with the Regional Tsetse and Trypanosomosis Control Programme, have shown that the distribution of tsetse flies, in particular *Glossina morsitans morsitans* and *G. m centralis,* have considerably expanded over the years and are further spreading into areas where livestock hosts are readily available. Reliable and detailed information on the tsetse distribution in Zambia is unavailable due to the lack of comprehensive survey or control programmes.

It was believed that goats were tolerant to trypanosomosis, but research findings have revealed that the animals are fully susceptible to infection with pathogenic trypanosomes[22,27]. Trypanosomosis has been demonstrated to occur in goats both under field and experimental conditions[7,12,20,21] and the economic impacts of the disease in goats can be substantial[6,13] .Nevertheless, trypanosomosis is one of the least studied diseases in Zambian goats as evidenced by the lack of appreciable information on trypanosomosis in the literature. One of the major problems encountered in the study of trypanosomosis in goats was that the trypanosome organisms are difficult to detect because of the low parasitaemia that occurs[2,19]. This resulted in a serious underestimation of the extent of the problem, leading to improper application of control measures[2]. Information on all aspects of the disease epidemiology under Zambian conditions is therefore indispensable in the battle against the disease. Effective monitoring of tsetse and trypanosomosis control requires reliable surveys of the distribution of the disease[9,24]. The main objective of this study was to establish the prevalence of trypanosomosis in goats in the study area so as to determine whether trypasomosis is a constraint to goat health in the area. All the goats used in this study came from the Luangwa-Zambezi Rift Valley area of Zambia.

The methods used were the haematocrit centrifuge (HCT) and polymerase chain reaction (PCR) techniques, which are highly specific and sensitive diagnostic techniques. Blood samples from the same goats were used for both HCT and PCR. For HCT, blood was spun in a haematocrit centrifuge to concentrate the trypanosomes at the buffy coat layer. This method is more effective in the detection of *T. congolense,* a strictly plasma trypanosome than *T. brucei* and its sensitivity is increased by concentrating the trypanosomes at the buffy coat by centrifugation. The technique has been reported to be sensitive and is the most widely used field test for the detection of animal trypanosomes[19,23].

Blood samples collected in EDTA tubes from 120 goats were used to screen for trypanosomes by haematocrit examination. A microhaematocrit capillary tube (75 mm long, 1–1.2 mm in diameter) was filled to the ¾ mark with uncoagulated blood and centrifuged for 5 min at 15 000 rpm/min to obtain the buffy coat. The tube was then cut just below the buffy coat region and a plasma drop with buffy coat contents was placed on a glass slide and covered with a cover slip. The preparation was examined under a light microscope (Olympus, magnification ×40). Trypanosome search was done in 100 microscopic fields. The determination of the trypanosome species was done by observing movement patterns of the parasites and relative size as described elsewhere[25].

The recent development of the nucleic acid-based PCR in the diagnosis of infections has provided a unique opportunity for the rapid and sensitive detection of specific microorganisms. The PCR technique overcomes the limits of sensitivity and specificity imposed by other methods and was also employed in this study to establish the prevalence of the trypanosomal infection in goats from Luangwa-Zambezi Rift Valley areas of Zambia.

PCR was used for screening trypanosome antigen for *T. congolense, T. brucei* and *T. vivax* to determine active infections in goats at the time of blood collection.

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Whole blood samples, which were collected from goats at the abattoir, were blotted on a filter paper. These blood spots were air dried and stored at –20 °C[4,15]. Trypanosome sample DNA was isolated from the dried blood spots by Chelex-100, an anionic resin, using modifications of the methods described by previous workers[11,26]. Dried blood spot on filter paper (Whatman no. 41) in about 5 mm^2 area was punched out and soaked in a 1.5 mℓ microcentrifuge tube which contained 1 mℓ of ice-cold 0.15 % Saponin (Sigma, UK) diluted in PBS (pH 7.2). The mixture was kept in ice for 10 min while occasionally mixing by gently inverting the tubes to allow the erythrocytes to detach from the filter paper in the manner described by others [4].

The mixture was then centrifuged at 10 000 rpm for 1 min to collect trypanosomes at the bottom of the tubes. The supernatant fluid was then discarded and, 200 µℓ of 5 % Chelex-100 Resin (Bio-Rad, UK) in distilled water was added to the cell pellet taking care that the Chelex beads were evenly distributed by gently mixing the Chelex solution while pipetting. The mixture was incubated at 56 °C for 15 min. in a water bath before heating in boiling water for 8 min. The Chelex was removed by centrifugation at 10 000 rpm for 1 min and 100 µℓ of the supernatant fluid was transferred to a fresh centrifuge tube to serve as the sample template DNA for PCR amplification[14]. For each template sample, 3 separate 0.5 ml vials were prepared for *T.congolense, T. brucei* and *T. vivax* amplification. The standard PCR amplification was carried out in 25 µℓ reaction mixtures. The reaction mixtures were prepared by adding 2 µℓ of Chelex-isolated template DNA, Ready-To-Go-Beads® (Amersham, Pharmacia Biotech, UK), 1 µℓ of the respective primers (Takara, Osaka, Japan) and 25 µℓ distilled water.

The Ready-To-Go-Beads® have been optimised for PCR reactions and contain buffer, nucleotides and *Taq* DNA polymerase. The beads were designed as premixed, pre-dispensed reactions for performing amplifications. The only reagents that need to be added to the reaction are template DNA and specific primers. This format significantly reduces the number of pipetting steps, thereby increasing the reproducibility of the PCR technique and minimising the risk of contamination (Instruction manual *Ready to Go* {PCR Beads}, Amersham Pharmacia Biotech, UK). When brought to a final volume of 25 µℓ, each reaction tube contained approximately 1.5 units of *Taq* DNA polymerase, 10 mM Tris-Hcl, (pH 9.0), 50 mM KCl, 1.5 mM $MgCl_2$, 200 µmol of each dNTP and some stabilisers (Instruction manual *Ready to Go* {PCR Beads}, Amersham Pharmacia Biotech, UK). The reaction mixtures were overlaid with 20 µℓ paraffin oil (Sigma, UK) and cycled in a programmable heating block (Astek Inc. Japan) as follows: samples were incubated at 94 °C for 3 min in an initial denaturing step and then were subjected to 40 cycles involving denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 1 min. The samples were then incubated at 72 °C for 7 min and then cooled to 4 °C to stop the reaction[14].

Two µℓ of DNA loading buffer (Takara, Osaka, Japan) were added to the PCR product and 10 µℓ were placed onto a well of 2 % agarose gel in 1 × TAE buffer (40 mM Tris acetate, 1 mM EDTA). In the 1st well, only 5 µℓ Biomarker® (Sigma, UK) were added. The product was then separated by electrophoresis in the 1 × TAE buffer at 100 V until the dye had covered half way up the gel after approximately 40 min. The gel was then stained in 0.5 µg/mℓ concentration of ethidium bromide for 7 min on a shaker and washed 3 times with PBS for 5 min per washing. The reaction fluorescent bands were visualised under UV light using UVP dual-intensity transilluminator (Japan). The primer sets used for repetitive nuclear DNA sequences of different trypanosomes species, subspecies and subgroups were referred to as reported sequences[14]:

TBR1 (CGAATGAATATTAAACAATGCGCAG), and
TBR2 (AGAACCATTTATTAGCTTTGTTGC) for *T. brucei brucei*

TCN1 (TCGAGCGAGAACGGGCACTTTGCGA), and
TCN2 (ATTAGGGACAAACAAATCCCGCACA) for *T. congolense,* savanna sub-group.

TVW1 (CTGAGTGCTCCATGTGCCAC) and

TVW2 (CCACCAGAACACCAACCTGA) for *T. vivax.*

Cyopreserved blood-stage trypomastigotes of *Trypanosoma congolense* were thawed and inoculated intravenously into 2 goats. Ten days later, 5 mℓ jugular blood was collected from each goat and *Trypanosoma congolense* parasite was demonstrated by buffy coat technique. After the confirmation of the presence of the parasite, a drop of the infected blood was blotted on filter paper (Whatman 41) to make blood spots for inclusion as a positive control to ascertain whether or not the PCR technique was detecting trypanosomes.

Table 1: **Trypanosome infection in Zambian goats.**

Species	Sample size	Methods	
		HCT (No. positive)	PCR (No. positive)
T. congolense	120	0	0
T. vivax	120	0	0
T. brucei	120	0	0
T. congolense	2 (Positive control)	2	2

The results of both the HCT and PCR analyses revealed no positive natural trypanosoma infections, but positive PCR results were obtained for the 2 goats inoculated with *T. congolense* (Table 1). This finding indicates that the PCR technique is effective and that none of the 120 goats examined was infected with *T. congolense, T. vivax* or *T. brucei* at the time of blood collection.

Given the small sample size used in the present investigation, it remains uncertain whether clinical trypanosomosis constitutes a serious threat to goat health in the areas where the animals originated. The samples were taken at a market, and the goats sampled are therefore unlikely to have included ones that were visibly sick. However, it has been reported elsewhere[25] that goats are rarely affected by trypanosomosis under field conditions, a suggestion apparently supported by another report[18]. According to an ILCA report[10], goats inherit a predisposition to trypanotolerance and then develop the tolerance through exposure. Lovelace *et al.*[17] considered Zambian Gwembe Valley goats to be unaffected by trypanosomosis because they thrive in areas where other livestock cannot be kept due to tsetse infestation. This reflects the perceptions of district veterinary officers who in a recent nation-wide questionnaire survey on goat diseases in Zambia rated trypanosomosis as one of the least serious in terms of occurrence[1]. In addition, it has been demonstrated that indigenous sheep and goats can be naturally infected with trypanosomes but there was no evidence that the infection caused mortality or decreased productivity[3]. However, in Zambian goats experimentally infected with *T. congolense,* decreased productivity and high mortality was reported[28]. In exotic sheep and goats, and crossbreeds, high levels of trypanosomosis, resulting in

mortality and decreased growth rates, are known to occur[5,8].

In spite of the limitations imposed by small sample size, this investigation suggests that trypanosomosis does not pose a major constraint to goat production under the existing extensive goat management practices in the areas where the animals originated.

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