DETECTION OF THEILERIA LESTOQUARDI DNA BY LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) IN GOATS IN MARAWI AREA, NORTHERN STATE, SUDAN

Mohammed O. Hussien[1]; Khalid M. Taha[2]; Ibrahim A. Ibrahim[1]; Abdelghafar M. Elfahal[1]; Khalid A. Enan[1]; Abdel Rahim M. El Hussein[2]

[1]Central Laboratory, Ministry of Science and Technology (MST), P.O. Box 7099, Khartoum, Sudan.
[2]Atbara Veterinary Research Laboratory, P.O. Box 121, Atbara, Sudan.
[3]Institute of Veterinary Research, Animal Resources Research Corporation, P.O. Box 8067 (Al-Amarat), Khartoum, Sudan.

المستخلص

اجريت دراسة لمعرفة نسبة الأصابة بثايليريا لستوكواردي (*Theileria lestoquardi*) باستخدام تقنية LAMP. من قري متعددة في منطقة مروي بالولاية الشمالية خلال شهر مايو 2008. أخذت 46 لطخة دموية في ورقة ترشيح من ماعز نوبي بالغ من الجنسين معافى ظاهرياً فى هذه القرى . استخلص الحمض النووي من اللطخات الدموية باستخدام طريقة الفينول كلوروفورم . من مجموع 46 حمض نووي مستخلص وجد ان 36 (3و78%) كانت موجبة للاصابة بثايليريا لستوكواردي (*Theileria lestoquardi*) باستخدام تقنية هذه الطريقة .

Abstract

In a study to detect *T. lestoquardi* infection using LAMP , 46 blood spots on filter paper were collected from adult apparently healthy Nubian goats of both sexes from several villages in Marawi area, Northern State, during May 2008. DNA was extracted from the blood spots using phenol chloroform method. Out of the 46 extracted DNA 36 (78.3%) were positive for *T. lestoquardi* infection using this technique.

Introduction

Theileria parasites are tick–transmitted, obligatory intracellular parasites that belong to the family *Theileriidae* and the order *Piroplasmida* (Levine, 1988). A minimum of six *Theileria* species infects small ruminants of which *T. separata*, *T. ovis* and *T. recondita* are nonpathogenic; whereas *T. lestoquardi*, *T. luwenshuni* and *T. uilenbergi* are pathogenic (Yin *et al*., 2007). In Sudan, *T. lestoquardi* is highly pathogenic for sheep. It causes malignant ovine theileriosis (MOT) and is mainly transmitted by *Hyalomma anatoliccum* ticks (Levine, 1973). *T. lestoquardi* infection in sheep is an emerging constraint to sheep industry in Northern Sudan (El Hussein *et al*., 1993; El Ghali and El Hussein, 1995; Ahmed, 1999). Although the disease was mentioned to affect small ruminants, it was particularly less studied in goats. The disease was successfully transmitted experimentally from infected sheep to goats via blood transfusion and infected tick application (Taha, 2000). In EdDamer Province, Northern Sudan, 10 out of 82 (12.2%) goats showed piroplasms in their blood. Moreover, antibodies against *T. lestoquardi* were detected in apparently healthy goats in River Nile State, Sudan (Taha *et al*., 2003). Generally, the clinical picture of the disease is mild in goats. Diagnosis of MOT is based on combination of the clinical signs and / or pathological findings with demonstration of parasitic stages in blood or impression smears of superficial lymph nodes and internal organs including liver and spleen. Several molecular–based methods for detection of ovine *Theileria* species have been developed. Of these, the rapid, simple and sensitive loop-mediated isothermal amplification (LAMP) technique was developed (Notomi *et al*., 2000) and applied for detection of ovine theileriosis (Liu *et al*., 2008; Salih *et al*., 2012).

Meagre data is available on animal disease situation in Marawi area of Northern Sudan. This area is envisaged as suitable for large schemes for small ruminants breeding for export purposes. This prompted us carry out this investigation on *Theileria lestoquardi* infection in goats as a possible carrier to parasite and play a role in epidemiology of MOT.

Materials and Methods

Study area

Marawi area (18° 29' 0" N 31° 49' 0" E) lies at an altitude of 905 feet above sea level, with desert climate where maximum ambient temperature may reach up to 42˚C and a minimum of 14˚C, relative humidity around 20% and rainfall is scanty. Sheep and goats in this area are raised in households for milk, meat and sale at times of necessity. They are usually housed in sheds made of local materials mostly tree branches and sometimes allowed to graze on grass and agriculture crop residues in contact along the River Nile banks.

Sample collection

The survey was carried out in compliance with the animal welfare code of Sudan. A total of 46 blood spots on filter paper (Whatmann # 3) from ear vein for DNA extraction were collected during May, 2008 from adult apparently healthy Nubian goats of both sexes from several villages in Marawi area, Northern State. The filter papers were individually sealed off in small polythene bags avoiding very carefully contamination.

Deoxyribonucleic acid (DNA) extraction

DNA was extracted from blood spots on filter paper using phenol chloroform method (Sambrook *et al*., 1989).

LAMP primers Mix

Primers mix contains four primers (FIP, BIP, F3 and B3) which were derived from the sequence of the *T. lestoquardi* clone-5 gene (DQ004500, Bakheit *et al*. (2006). Final concentration of FIP and BIP is 40 pmol each, F3 and B3 is 5 pmol each. The sequences of each primer are shown in Table 1.

Table 1: Nucleotide sequences of LAMP primers for detection of *T. lestoquardi* based on the clone-5 sequence.

Primer name	Type	Length	Sequence (5′-3′)
F3	Forward outer	21 bp	AGATACCAAGGAAACTGA
B3	Backward outer	24 bp	TGTATCCTTAGGTTTTTC
FIP	Forward inner primer	49 bp	CAGGAGAAATAGGAGTTTCAGG TTCCAAAGGATAAGAAAGATGAAAAGG
BIP	Backward inner primer	44 bp	GTATCGCACCAGAACCTCAACAC AGTTTCTTCTTTATCCTGATC

LAMP condition

The reaction was performed in a final volume of 25 µl which contained 12.5 µl 2x LAMP reaction buffer (40 mM Tris-Hcl (pH 8.8), 20 mM KCl, 20 mM $(NH_4)_2SO_4$, 16 mM $MgSO_4$, 0.2% Tween 20 and 1.6 M Betaine), 8 U of *Bst* DNA polymerase, 40 pmol each FIP and BIP primers, 5 pmol each F3 and B3 primers and 2 µl of target DNA. The mixture was incubated in a heat block at 63^{o}C for 45 minutes and then 80^{o}C for 2 minutes. The LAMP products were subjected to electrophoresis on a 1.5% agarose gel containing 0.5 µg/ml ethidium bromide and visualized under ultraviolet light.

Results and Discussion

Out of 46 examined goat DNA extracted from blood spots on filter paper 36 (78.3%) were positive for *T. lestoquardi* infection using LAMP technique in Marawi area, Northern State (Fig.1).

To the best of our knowledge, this is the first report showing *T. lestoquardi* infection in goats in Marawi area, Northern State and for application of LAMP technique under field conditions. Mohammed and Salih (2003) reported a clinical case of theileriosis in a local goat in Red Sea State (Eastern Sudan). Recently, an outbreak of malignant ovine theileriosis that caused high loss among imported foreign goats in Atbara Town, Northern Sudan was documented (Taha *et al*., 2011). In the current report, the high prevalence rate of *T. lestoquardi* infection in goats (78.3%) may strengthen

the hypothesis that local goats may be only carrier to parasite and hence play a role in epidemiology of MOT. This also highlights the hazards imposed by this parasite on any large scale breeding or genetic improvement programs of foreign goats or their crosses and / or sheep in Marawi area.

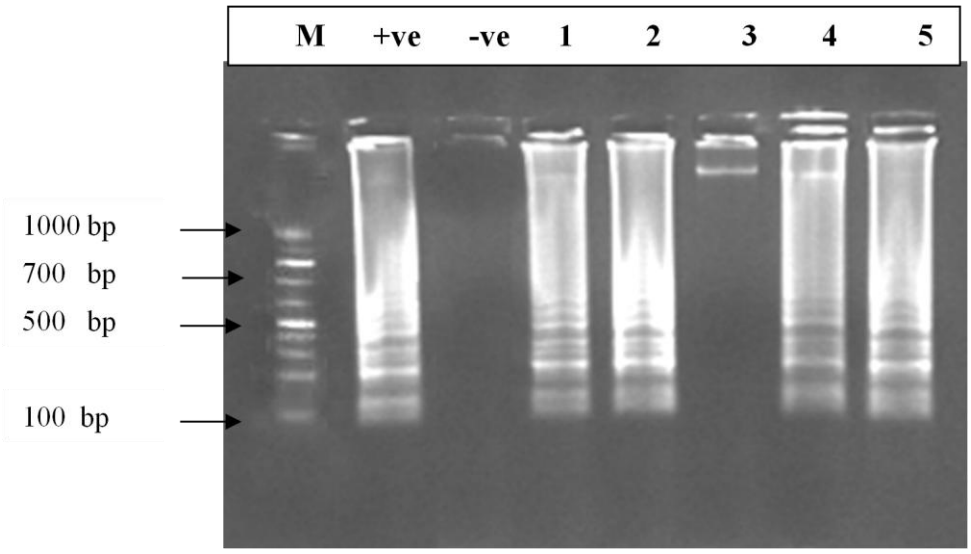

Fig. 1. Agarose gel (1.5%) electrophoresis showing detection of *T. lestoquardi* by LAMP technique in DNA extracted from caprine blood spots. *M* = marker, +ve positive control, -ve negative control, 1-5 goats samples.

Conclusion

Construction of the new Marawi dam and the expected increase in agriculture and animal production activities in this area may create an ideal environment for vectors and disease transmission. Further investigation on malignant ovine theileriosis in the Northern State is required before embarking upon large schemes for small ruminants (sheep and goats) breeding

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