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Comparison of Primer Sets for Amplification of 30 kDa Merozoite Surface Antigen of Bovine Theileriasis

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Abstract: The efficiency of two primer sets that used for diagnosis of *Theileria annulata* infection in cattle were evaluated in 28 cows that showed clinical signs of theileriosis. Both conventional method and Polymerase Chain Reaction (PCR) were used to confirm theileria infection. For PCR, two primer sets that amplify the 30 kDa major merozoite surface antigen gene were used. Only 12 cows (42.85%) were positive for theileria infection with blood film. Higher number of cows were diagnosed positive by using PCR, the number of positive cows using primer set one (N516/N517) was 21 cows (75%) and by using primer set two (Tams1F/Tspm1R) was 27 (96.42%). It could be concluded that primer set Tams1F/Tspm1R is the first choice when diagnosis of *Theileria annulata* is decided.

Key words: *Theileria annulata*, cow, gene, primer, blood film, Egypt

INTRODUCTION

Tropical theileriosis is one of the most prevalent and economically important disease of cattle (Mirzaei, 2007). The principle causative agent of bovine theileriosis is the protozoan parasite *Theileria annulata* and transmitted by the ticks of the genus *Hyalomma*. The disease is observed in South Europe, North Africa, middle and South Asia and the Middle East and threatens approximately 250 million cattle (Young, 1981; Viseras *et al*., 1997; Salih *et al*., 2007). Theileriosis causes serious economic losses through mortality and loss of productivity (Glass *et al*., 2003; Aktas *et al*., 2004).

Cattle with theileriosis usually showing fever, enlargement of superficial lymph nodes that drain the area infested with infected ticks (Radostitis *et al*., 2006). In the early phase of parasitemia it can be easily diagnosed by Giemsa stained thin blood film from peripheral blood or by preparing lymph smear stained by Giemsa from enlarged lymph nodes (Aktas *et al*., 2006). Serological tests are best suitable for the diagnosis in later phases of the disease and in carrier animals in which the antibody titers are usually higher and the piroplasm parasitaemia drops to microscopically undetectable levels (Bakheit *et al*., 2004). Polymerase Chain Reaction (PCR) is considered the most sensitive and specific test for detection of theileria infection (De Kok *et al*., 1993; D'Oliveira *et al*., 1995; Jongejan *et al*., 1995; Aktas *et al*., 2006) which amplify the 30 kDa major merozoite surface *antigen* gene (Kirvar *et al*., 2000; Altay *et al*., 2007; Guzel *et al*., 2008). Two primer sets are commonly used for diagnosis of *Theileria annulata* infection; Tams1F/Tspm1R (Altay *et al*., 2007) and N516/N517 (Azizi *et al*., 2008). The two primer sets were used in separate studies but no study of them compared the efficiency of the two primers in detection of *Theileria annulata* infection which is the aim of the present study.

MATERIALS AND METHODS

Animals: A total number of 28 cows were subjected to the study. Animals were presented to the Veterinary Teaching Hospital, Assiut University showing clinical signs of theileria infection, the clinical signs were high fever 40-41.5°C, enlargement of one or more superficial lymph nodes, infestation with ticks, ocular discharge and corneal opacity.

Samples

Blood smear: Blood sample was collected from the ear vein and used for making blood film according to Coles (1986). Thin blood film was fixed in absolute Methyl alcohol and stained with Giemsa stain and examined under the Oil immersion lens to detect the piroplasms or trophozoite.

Whole blood samples: Whole blood samples were collected from the jugular vein in vacutainer tube

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Table 1: Oligonucleotide primers used to amplify 30 kDa of *Theileria annulata*

Primers	Sequence	Amplicon size
Primer set 1		
N516F	GTAACCTTTAAAAACGT	721 bp
N517R	GTTACGAACATGGGTTT	-
Primer set 2		
Tams1F	ATGCTGCAAATGAGGAT	785 bp
Tspm1R	GGACTGATGAGAAGACGATGAG	-

containing EDTA as anticoagulant and preserved directly at -20°C till DNA extraction. DNA extraction was carried out using commercial kits (QIA amp blood kit, Qiagen, Ltd, UK).

PCR reaction: Two primer sets were used to amplify the 30 kDa major merozoite surface *antigen* gene (Table 1). PCR was performed in 25 μL reaction mixture containing 12.5 μL Master mix. In liquid form (20 mM Tris-HCL PH 8.4, 50 mM KCL, 0.1 mM each of the four dNTPs d ATP, d CTP, d TTP, d GTP, 1.5 mM of $MgCl_2$, 1.5 units of RED Taq DNA polymerase), 5.5 μL Dnase, Rnase free water, 50 pmol primer and 5μL of DNA sample.

Thermal cycling for primer set one (N516/N517) was 94°C for 5 min, followed by 37 cycles consisting of 1 min at 94°C, 1 min at 50°C, 2 min at 72°C and final extension step at 72°C for 10 min for primer set two (Tams1F/ Tspm1R) was 94°C for 5 min, followed by 37 cycles consisting of 1 min at 94°C, 1 min at 55°C, 2 min at 72°C and final extension step at 72°C for 10 min. In addition, positive control was DNA extract from theileria infected cow that was positive by blood film and by using the two sets of primers. Negative control that contains all the PCR reaction mixture except the DNA template was also used. The thermal cycling was performed by means of Thermocycler (TECHNE TC-312). The amplification product was separated on 2% agarose gel electrophoresis and visualized by ethidium bromide staining. The 100 bp DNA ladders were used as DNA molecular weight standards. The result obtained through High performance Ultraviolet Transilluminator (UVP, INC, UK).

RESULTS AND DISCUSSION

From the 28 examined cows, only 12 (42.85%) cows were positive for theileria infection with blood film. Higher number of cows were diagnosed positive by using PCR (Fig. 1), the number of positive cows using primer set 1 was 21 cows (75%) and by using primer set two was 27 (96.42%). Results for individual cases are shown in Table 2. Number and percents of positive cases are shown in Table 3. The aim of the present study was to compare the efficiencies of the PCR method with the different primers used to detect the 30 kDa major merozoite surface

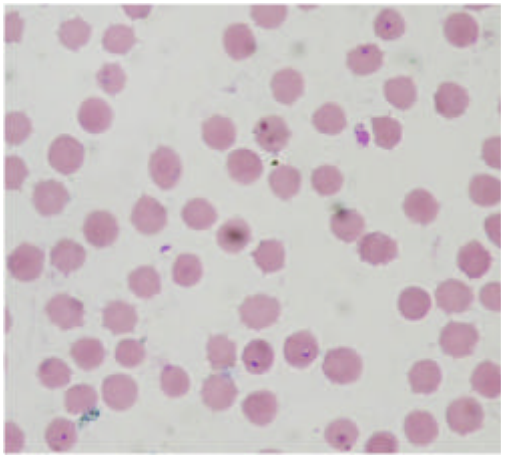

Fig. 1: Higher number of cows diagnoseed positive by using PCR

Table 2: Comparison of conventional and PCR method for diagnosis of theileria infection

	PCR	
Conventional (blood film)	Primer set 1 (N516F/N517R)	Primer set 2 (Tams1 F/Tspm1 R)
-	+	+
-	-	+
+	+	+
-	+	+
-	+	+
+	+	+
+	+	+
+	+	+
+	+	+
+	+	+
+	+	+
+	+	+
+	+	+
-	+	-
-	-	+
-	-	+
-	+	+
-	-	+
-	-	+
+	+	+
-	+	+
+	+	+
-	+	+
-	+	+
-	-	+
-	+	+
+	+	+
-	-	+
+	+	+

Table 3: Number and percent theileria infected cows using conventional and PCR methods

PCR					
Conventional (blood film)		Primer set 1 (N516F/N517R)		Primer set 2 (Tams1 F/Tspm1 R)	
Positive (No.)	%	Positive (No.)	%	Positive (No.)	%
12	42.85	21	75	27	96.42

antigen gene of *Theileria annulata*. Early phase of parasitemia can be easily diagnosed by detecting the trophozoit in peripheral blood, negative results of microscopic examination of blood films do not exclude latent infection (Aktas *et al*., 2006).

Polymerase Chain Reaction (PCR) offers important advantage such as the greater sensitivity and specificity over conventional techniques in detecting both piroplasm-infected and carrier animals. The number of the positive cases detected by primer set one (N516/N517) 21 cows (75%) was lesser than positive cases detected by primer set two (Tams1F/Tspm1R) 27 (96.42%). It is clear from Table 2 that all cases that were positive with blood film give positive results with the two sets of primers. Some negative cases with blood film were negative with primer set one and positive with primer set 2.

CONCLUSION

It could be concluded that primer set Tams1F/Tspm1R is the first choice when diagnosis of *Theileria annulata* using PCR is decided.

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