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MOLECULAR AND HEMATOLOGICAL STUDY OF *TOXOPLASMA GONDII* IN HORSES

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ABSTRACT : Limited information about prevalence of *Toxoplasma gondii* infection in equine around world. The objective of this investigation was to estimate the frequencyof *T. gondii* infection in equinewithin Baghdad city, as well as to study the hematological changes associated with infection. Blood samples collected from 40 horses were examined for *T. gondii* using highly specific and sensitive Reverse transcriptase, (RT-PCR) between first of October 2018 to March 2019. Hematological parameters in whole blood samples were also examined. Overall occurrence of *T. gondii* genotype UPRT1 was 25% in horse, a significant increase was detected in younger horses (28.5%) compared to aged 16.6%. On the other hand, the increase observed failed to reach the level of statistical significance in 26.6% male than 20% female. In infected groups means of RBC7.20 ± 0.41/mm^3, WBC5.88±0.44/mm^3, Granulocytes 3.52±0.28/mm^3, Monocytes 0.802 ± 0.05/mm^3, Lymphocytes 1.60 ± 0.17/mm^3, PCV36.25 ± 1.03% and Hb 11.39 ± 0.43 g/dl were with a non-significant differences when compared to control group RBC 7.98 ±0.33/mm^3, WBC 5.92 ± 0.25/mm^3, Granulocytes 3.32 ± 0.22/mm^3, Monocyets 0.99 ± 0.15/mm^3, Lymphocytes 1.73 ± 0.10/mm^3, PCV 39.25±1.67%, HB12.66 ± 0.54 g/dl.DNA of *Toxoplasma gondii* was detected in 25% of blood samples in horse examined suggests a risk of foodborne disease associated with horse meat consumption.

Key words : *Toxoplasma gondii*, horse, polymerase chain reaction (RT PCR), Gene expression, blood count.

INTRODUCTION

Horses useful and important animals to human being in different ways, such sports, police works, carriages, so on. Horses meat are also the popular and delicate food for people in the world (Miao *et al*, 2013). Worldwide the incidence of *Toxoplasma gondii* in naturally infected horses may be up to 80% (Tassi, 2007) and the infection consider subclinical, a typical signs includes fever, ataxia with retinal degeneration, encephalomyelitis and stillbirth in pregnant equines or abortion, toxoplasmosis cases in human associated with eating infected horses meat have been reported in some area (Ioana Pas Anamaria, 2015).

Toxoplasma gondii is an obligate intracellular protozoan parasites, in the phylum Apicomplexa, a significant veterinary, human pathogen. Enters the hosts through gastrointestinal tract and isassociated with a risk for congenitally infected infants (Montoya and Liesenfeld, 2004), fatal infection of the fetus during pregnancy (Havelaar *et al*, 2007) can result in noteworthy reproductive failures, public health and economic loss, since eating of infected meat could facilitate transmission (Faria *et al*, 2007). In addition to the role of cytokines, humoral and cell mediated immunity stimulates during toxoplasmosis infection (Aljanaby, 2012).

Three types of strains for *T. gondii*, type one (virulent), two and three (low virulence), which all can infect humans as well as animals, especially in rural areas, due to the habits for acquiring the disease (Aljanaby, 2012; Black and Boothroyd, 2000). It was record in Iraq (AL-Fertosi and Juma, 2006).

Global gene expression studies to clarify transcriptomes unique with tachyzoite in addition to oocyst of *T. gondii* development (Manger *et al*, 1998; Fritz *et al*, 2012; Buchholz, 2011). *T. gondii* differentiates between developmental stages, the toxoplasma transcriptome is cell cycle regulated it is uncertain how the parasite senses changes in its environment to trigger different in its transcriptome. To now, 2-component regulatory systems for triggering transcriptional responses to extracellular environment changing have not been described for *T. gondii* (Sini Skariah and Dana G Mordue, 2012).

MATERIALS AND METHODS

Sample collection

During the period from October 2018 to March 2019 blood samples were collected from 20 horses by veterinary practitioners in different regions of Baghdad city, horese ages ranged between (4-18) years with consideration of sex. Each blood sample was mixed with EDTA for more analysis.

Gene expression

RNA extraction : Direct-zol™ RNA MiniPrep, R2051, ZYMO RESEARCH / USA). The Direct-zol™ RNA MiniPrep provides a streamlined method to purify of up to 50 µg '*per prep'* of high quality RNA directfrom sample inTri reagent. Total RNA include the small RNAs, 17-200 ntis effectively isolated from different sample origin (blood).

Real-Time PCR (one-step RT-qPCR)

Toxoplasma DNA amplification have been done with specifically UPRT1 (EXT) of gene (Forward 5'-TCCACAGGGCTTCTAAAAT- 3' Reverse 5'-GAGTTGAGAACAGGCTTCAG- 3') by the KAPA SYBR FAST one-step qRT-PCR kit, Canada. The appropriate volumes of qPCR master mix, template and primers were transferred to each well of a PCR tube/ plate. The reaction tube/plate was caped and centrifuged briefly. Performing One-Step qRT-PCR.

The amplification accuracy of the presence of *T. gondii* genotype UPRT1 (EXT) of gene product was noticed by the value of cycle threshold (Ct) for the triplicate reactions.

Hematological study

Blood samples (Anticoagulated) were used to count the total leukocyte, Monocytes, Lymphocytes Granulocytes and RBC, Concentration of Haemoglobin (Hb), packed cell volume, PCV. Differential count was performed by using Humacount 60ts Three Part Hematology analyzer, Germany.

Statistical analysis : According to SAS (2012).

RESULTS

Results of RTPCR

Overall percentage of *T. gondii* prevalence was 25% in horses using RT-PCR. This indicated that there is a rate of correlation ($P<0.05$) between *Toxoplasma* infection and gene expression. The prevalence was lower in aged horse 16.6%, compared to younger animals 28.5% by using RT-PCR.

Based on gender, the incidencewas higher in males 26.6% compared to female animals 20% using RT-PCR with a non-significant difference (Table 1).

Hematological analysis

Concerning the effect of toxoplasmosis on hematological findings, the results in Table 2 demonstrate that there is a non-significant differencein the means of RBC, WBC, Granulocytes, Monocytes, Lymphocytes/ mm^3 and PCV% Hb (g/dl) in infected groups compared with control.

DISCUSSION

In the present survey, using RT-PCR, *T. gondii* overall prevalence was 25% in horses, *Toxoplasma* DNA was detected in 43% of horse meat samples (Aroussi Abdelkrim *et al*, 2015), while no DNA *T. gondii* was found in any of heart samples collected from slaughtered horses (Ioana *et al*, 2015). While, prevalence of *T. gondii* was 17.65% using RT-PCR in blood sample (Ibrahim Hany, 2017).

Differences in climates, hygiene conditions may beeffected on different prevalence results and the prevalence of *T. gondii* in catsfinal host (Miao Qiang *et al*, 2013). PCR is the molecular technique detection, to acute infection as its reduces in chronic infections because

Table 1 : Prevalence of toxoplasmosis among horse by using RT PCR.

Variables Toxoplasmosis	Total 40	Real-time PCR, UPRTF2 10 (25%)	Chi-square-χ^2 (P-value)
Age group (year)			
10 or less	28	8 (28.5%) *	4.52 * (0.046)
>10	12	2 (16.6%)	
Sex			
Male	30	8(26.6%)	2.037 NS (0.092)
Female	10	2(20%)	

* ($P < 0.05$) in aged group 10 years old or less compared to those more than 10 years old.

Table 2 : Hematological parameters level in horse.

Toxoplasmosis	RBC	WBC/mm3	Granulocytes	Monocytes	Lymphocytes	PCV %	Hb (g/dl)
Infected (10)	7.20 ± 0.41	5.88 ± 0.44	3.52 ± 0.28	0.802 ± 0.05	1.60 ± 0.17	36.25 ± 1.03	11.39 ± 0.43
Control (30)	7.98 ± 0.33	5.92 ± 0.25	3.32 ± 0.22	0.99 ± 0.15	1.73 ± 0.10	39.25± 1.67	12.66 ± 0.54
LSD value	1.341 NS	1.056 NS	0.882 NS	0.561 NS	0.435 NS	6.29 NS	2.09 NS

NS: Non-Significant.

DNA of *Toxoplasma* will not be present in samples especially, blood samples (Boothroyd, 2009), as well in tissues there is a non-homogeneous distribution of *T. gondii* tissue cysts (Jurankova *et al*, 2013).

Relationship between ages, gender was performed by comparing the prevalence of *T. gondii* in horse and was higher in younger 28.5% than aged populations 16.6% significantly may be due to the variable of physiological and immunity state in horse infected with toxoplasmosis.

The current result disagreement withthe reports in Libya (Al-Mabruk *et al*, 2013), Tunisia (Boughattas *et al*, 2014; Lahmar *et al*, 2015), Egypt (Younis *et al*, 2015). Aged horses >10-year old were more to be positive than horses under 10 years old, the increase in risk correlation with increase of age due to longer contact with infective oocysts from environment, increasing the risk of stock contamination (Boughattas *et al*, 2011; Santana Luís Fernando *et al*, 2015).

The current study showed increase prevalence in male 26.6% when compared to female 20% with a non-significant difference using RT-PCR, male horses are more sensitive to the infection by the parasite than female (Boughattas *et al*, 2011), while (Ioana Pas Anamaria *et al*, 2015) reviled no significant differences between age and gender of animals.

The prognosis of recovery in affected horse can made with laboratory and clinical data like hematological & blood biochemical parameters (Matanovic *et al*, 2007), polymorphonuclear leukocytes are first t arrive to the infection region (Jackson *et al*, 1995). Neutrophil elicited and appear within few minutes of chemokine release bytachyzoite (Denney *et al*, 1999). Neutrophils are very important for controlling infections in mice (Bliss *et al*, 2001; Del Rio *et al*, 2001), humans (Bliss *et al*, 1999).

In infected group, present result showed that neutrophil was slightly increased compared to control. But, lymphocytes was slightly decreased in infected horse, neutrophil was found to be higher in toxoplasmosis of gerbils (ATMACA Nurgül *et al*, 2015), increase neutrophils probably an inflammatory responses to the tachyzoite multiplication during infection period. Tonin *et al* (2013) reported that the numbers of lymphocytes were in high counts in *T. gondii* rodents, but other studies found that the blood parameters remains stable and not changeable during period of infection (Lappin, 1996). This variance may be related to the different sampling periods of infections of all those different studies.

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