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Research Article

EVALUATE MICRONUCLEI IN THE PERIPHERAL BLOOD LYMPHOCYTES OF SARCOCYSTOSIS INFECTED EWES

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ABSTRACT

The purpose of the study was observed the DNA distraction in the peripheral blood lymphocytes of sarcocystosis infected ewes by micronuclei assay. Forty sarcocystosis infected ewes and twenty uninfected as a control group were involved in this study. Lymphocytes from peripheral blood of infected and un infected ewes was used for culture and subsequent cytogenetic studies were performed. The results showed that the MNmean of infected ewes was significantly ($p \leq 0.05$) higher 32.05 ± 0.95 than the uninfected group 11.95 ± 0.622. These findings indicated of an increase in DNA damage in infected ewes measured by MN frequency.

Keywords: Sarcocystosis, Cytogenetic analysis, Micronuclei assay, lymphocytes, ewes.

INTRODUCTION

Sarcocystosis is an a worldwide zoonotic disease caused by obligatory intracellular protozoan parasite , man and herbivorous animals act as intermediated host while final host include human and predation animals, like dogs and cats, contaminated environment by shedding 200 million oocysts through patent period infection via faces (Nourollahi Fard et al., 2009 and Motamedi et al., 2011). Acute sarcocystosis in intermediate hosts can be characterized by encephalitis, inflammation of the brain and spinal cord, bleeding diathesis, it many cause fetal death and abortions of pregnant animals (Casparia et al., 2010). Mild and chronic sarcocystosis leads to a decrease in body weight and amount of fur (Tenterb et al., 1995). The micronucleus (MN) assay is an excellent biomarker candidate, since it is able to detect chromosome breakage or malfunction of mitotic spindle caused by aneugenic mechanisms (Kashyap and Reddy, 2012). MN arise during mitosis when lagging chromosomes are left out the metaphase plate because mitotic defects (aneuploid effect) or when chromosome fragments lacking a centromere are not captured by spindle microtubules (clastogenic effect); in both cases the genetic material was unable to be incorporated into the nucleus of daughter cells (Schmid ,1975). Aneuploid effect from the clastogenic damage Can be distinguished the by differences in MN size or by the a centromere presence (Afshari et al., 1994 and Migliore et al., 1996). Frequency aneuploid can be increased under exposition to certain endogenous or exogenous agents (Ramos-Remus et al., 2002). In the present study, micronucleus test was used for investigate of effect of sarcoystosis on peripheral blood lymphocytes of infected ewes for the first reported in Iraq.

MATERIALA AND METHODS

One gram from skeletal muscles of forty infected and twenty uninfected ewes, (Schmid, 1975). Trichinoscopy test was used to isolate positive samples from negative samples which considered as control group. Each sample was crushed between two clean glass slides followed by microscopic examination (x10 and x20) for the diagnosis of Sarcocystosis.

Micronucleus (MN)assay was done according to previous studies (Fenech et al .,2003 and Fenech ,2005), in which the number of micronucleated lymphocytes can be determined 1–2.5 ml sample of peripheral blood from jugular vein from each infected and uninfected was added to 5 ml of chromosome medium supplemented with 5 µg/ml of phytohaemagglutinin (PHA). The cultures were incubated for 72 hs at 37 °C. After 44 hs, 4.5 µg/ml of cytochalasin–B was added to each culture to block cytokinesis. Upon completion of the incubation, each cell culture was then subjected to hypotonic treatment by adding 8 ml of 0.075 M KCl and maintained for 7 min at 37 °C. Three repetitive fixations of each cell suspension were then performed with Methanol/Acetic acid (3:1, v/v). An aliquot of each cell suspension was then dropped onto a cold slide. The slides were air–dried and kept at room temperature for 1 day after which they were stained using acridine orange method.

The frequency of cells with 2 or more micronuclei was determined after assessing between 800 and 1900 binucleated cells from each cell culture. The slides were scored using the following scoring criteria of (Fenech et al .,2003 and Fenech ,2005); the diameter of the MN should be less than 1/3 of the main nucleus, MN should be separated from or marginally overlap with main nucleus as long as there is clear identification of the nuclear boundary and MN should have similar staining as the main nucleus.

All data were submitted to statistical analysis using T-test and by using SPSS program. P value < 0.05 was considered as statistically significant at ($p \leq 0.05$) (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

The result showed that microcystic cyst of ovine Sarcocystosis in skeletal muscle was 40 out of 60 (66.6%) was diagnosed by using trichinoscopy test. The cysts are cylinder shape and having pointed ends in the muscles that crushed (Figure1). The results reflected the high rate of infection with ovine Sarcocystis which agreed with previous study in Baghdad area (AL- Delemi ,1992).

The results showed that the Micro Nuclei (MN) mean number of infected ewes was significantly ($p \leq 0.05$) higher 32.05± 0.95 than that of and uninfected grou p 11.95±0.6229 (Table, 1and Figure 2, 3, 4 and 5).

Table1: The Means and SE of Micronucleus in Ewes

Ewe	No. of examined cells	Distribution of MN in cell				No. of cells contents on MN	No. of MN	No. of MN/Total cell no.
		0	1	2	3			
Sarcocystosis infected ewes **(40)**	1000	978.2 ± 0.60 B	13.63 ± 0.38 B	5.33 ± 0. 45 A	2.65 ± 0.14 A	21.45 ± 0.52 A	32.05 ± 0.95 A	0.032 ± 0.0009 A
Control (20)	1000	991.55 ± 0.38 A	35.85 ± 0.28 A	2.11 ± 0.19 B	0.55 ± 0.15 B	8.45 ± 0. 38 B	11.95 ± 0.622 B	0.0116 ± 0.0007 B

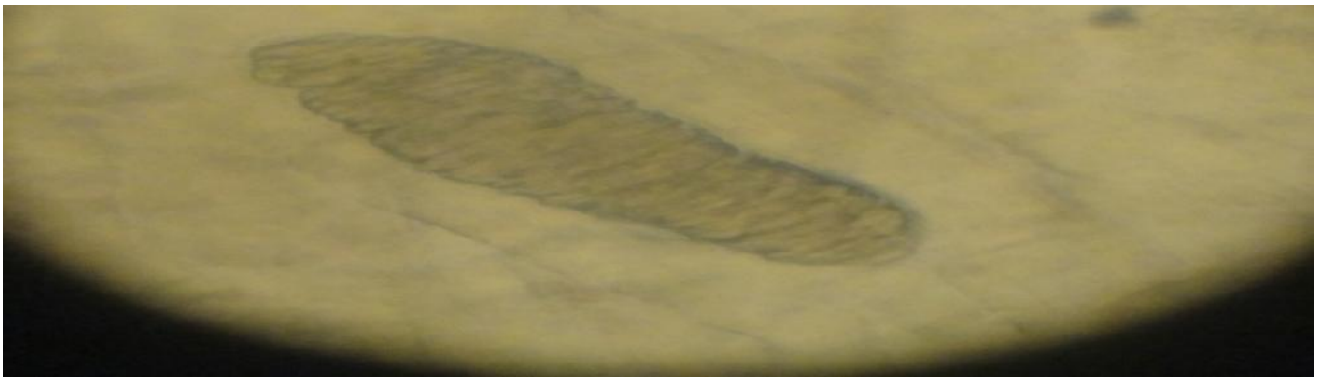

Figure1: Showing microcytic cyst of ovine sarcocystosis in skeletal muscle(X20)

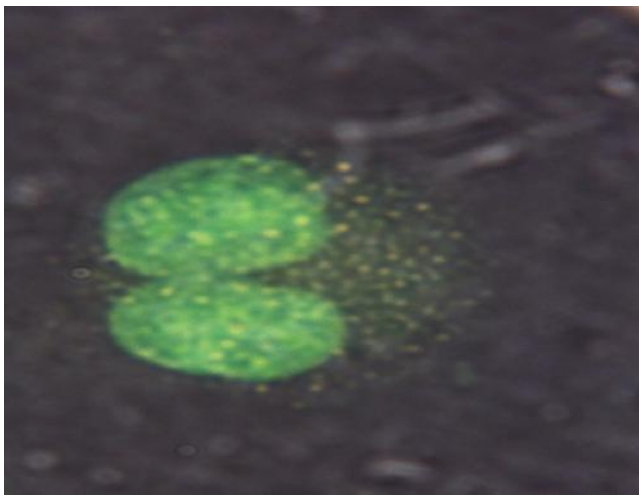

Figure 2: Normal Binucleated cell of control ewes (acridine orange staine X100).

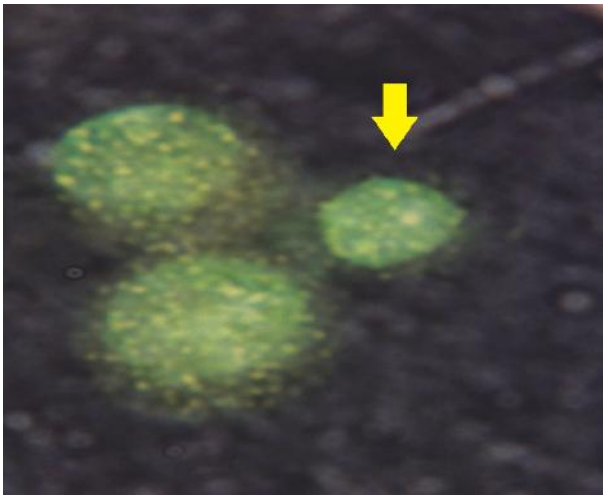

Figure 3: Binucleated cell with one Micronucleus of infected ewes (acridine orange staine X100)

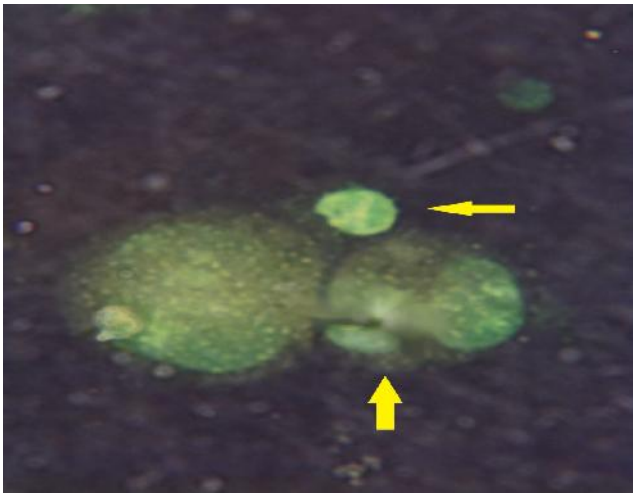

Figure 4: Binucleated cell with two Micronucleus of infected ewes (acridine orange staine X100).

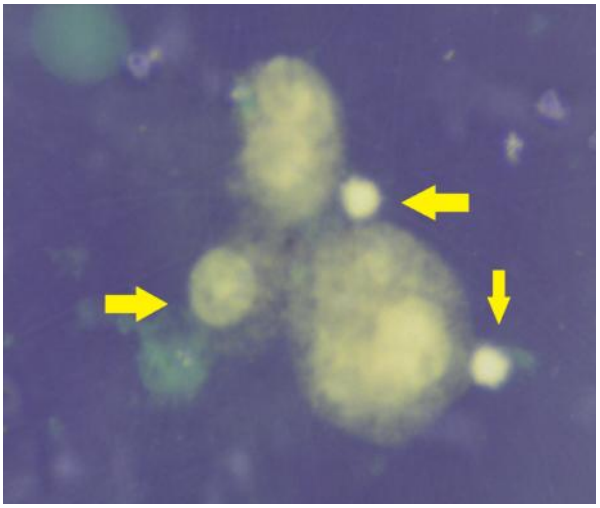

Figure 5: Binucleated cell with three Micronucleus of infected ewes (acridine orange staine X100).

Micronucleus test is one of the more common assays that were used for determination the damage in DNA (Lambertti et al.1983). The low cost and high reliability of the MN assay, has contributed to the world wide success and adoption of the biomarker for in vivo and in vitro studies of genome broke down (Bonassi et al,.2000) . Genomic damage can be occurs by environmental exposure to genotoxic factors,pathogen, medical technique (chemicals and radiation), deficiency in folic acid due to micronutrient, lifestyle (smoking, , drugs, alcohol and adverse condition), and genetic defects, such as transmitted defects in DNA repair and DNA metabolism (Bonassi et al .,2011). The results of our study showed that micronuclei frequencies were significantly ($p \leq 0.05$) higher in infected ewes compared with uninfected group; such results were shown in S. haematobium infection (Tohda et al., 1980). Also it was observed in Toxoplasma in which the host cells may reflect change the efficiency of cells for incorporation in cellular DNA (Hussein , 2014). There is evidence indicating that Schistosoma infection is involved in the incidence of several cancers. Urothelial cells collected from patients infected with S. haematobium were shown to have an increased frequency of micronuclei suggesting the induction of chromosome injury (Fouad et al ., 2002).

This study showed significant increase in MN mean number in sarcocystosis infected group than of uninfected group , which may be related to the parasite metabolism, in which certain chemicals might be released that might have a direct effect on the chromosomes.

CONCLUSION

The present study conducted the Sarcocystis has an effect on lymphocyte proliferation, and on the genetic material of the lymphocyte which caused a significant increase in Micro Nuclei mean of the infected group when compared to healthy controls group.

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