Plant Archives Vol. 19 No. 2, 2019 pp. 4015-4018 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

DETECT DNA DAMAGE IN LYMPHOCYTES OF BUFFALOES INFECTED WITH BRUCELLOSIS BY COMET ASSAY TECHNIQUE

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Abstract

The objectives of the present study is to assessment the effect of brucella on DNA damage in peripheral blood lymphocytes of water buffalo (*Bubalus bubalis*) by using comet assay technique. Forty blood samples (25 from infected buffalos and 15 from non-infected buffaloes) were collected to confirm the infected from non with rose Bengal test and to detect the effect damage of Brucella infection on DNA of lymphocyte cells by using technique of comet assay. The blood samples were grouped according to age of examining animals into two groups; under 4 years (≥ 4y) and over than4 years (< 4y). The DNA damage was exhibited by the comet assay method by showing the changes in tail length, tail moment and the % DNA in tail, was affected significantly in infected group when compare with the negative control group, in addition these DNA damage indicators in infected group were significantly increased with age.

In conclusion, *Brucella* infection in buffaloes caused marked damage of DNA of lymphocytic blood cells and this affection increased with age.

Key words: DNA damage, comet assay, brucellosis in buffaloes, lymphocytes.

Introduction

Brucellosis is generally an infectious bacterial disease caused by small, gram-negative, facultative aerobic organism of genus *Brucella*, affecting different mammals, including humans in many countries (Young, 1983 and 1995). Brucella infection in domestic buffalo from Species (*Bubalus bubalis*) which is caused by (*Brucella abortus*) (Fosgate, *et al.,* 2011) and the disease was transmission among different animals species (Dawood, 2008). After entry of the host, *Brucella* organisms first localize in regional lymph nodes, where they proliferate within reticuloendothelial cells, subsequent entry into lymphatics and development of bacteremia allows localization of the bacteria in a variety of tissues (Radostits, *et al*., 2007) and these organisms which are intracellular pathogens product cell toxicity by inducing cell apoptosis and altering of cell membrane (de Bagu¨e´s, *et al*., 2004). So, it maybe related to improved to free radical production, oxidative stress and the antioxidant depletion maybe concerned in the pathogenesis of brucella (Kataria *et al*., 2012). The single cell gel-electrophoresis (SCGE) test, or comet assay, is a sensitive, rapid and the reliable technique which can identify (DNA) single strand breaks (SSBs) as initial damage. It is a modification of the protocol firstly describe by Singh and his co-workers in 1988. (Singh, *et al.,* 1988). Using a program to analyze the comet image, three criteria for measuring DNA damage was tail length., (% DNA) in tail and tail moment. The extent of DNA damage was determined, via measuring the dispersion between the nucleus (comet head) in addition the resulting tail. At least 50 random cells must be analyzed for each sample. Quantitative estimation was performed using the image analysis comet level, and the analysis program that appeared calculated different parameters for each comet. Three parameters indicating DNA migration and tail length (distance from head to tail end) were estimated (average tail) induced in considered both (% DNA) in the tail and migration genetic material just as relative amount of DNA, In the tail. (Peggy, *et al*., 2006). In this technique a damaged cell has appearance of comet with tail regions and head. It is usually that SCGE made under of alkaline conditions the primarily detects of alkali-labile sites in DNA and SSBs (Tice *et al*., 2000). However, the advantage of SCGE are showing DNA damage in individual and small numbers

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of cells and providing a quantitative index for DNA damage (Mitchelmore and Chipman, 1998). Many samples including peripheral blood, buccal mucosal cells, cultured cells, solid tumor, sperm, cancer cells, bacteria, yeast cells, can be exposed to SCGE (Collins, 2003). This study had been assessment the effect brucellosis-diseaseon (DNA) damage in the buffalo (peripheral blood lymphocytes-PBL) by using of comet assay technique.

Materials and Methods

This study was carried out between December 2018 and February 2019 in Al-Qasim city. A total of forty (40) buffalo female were examined in current study, twenty five buffalo were with a history of abortion and marked emaciation and it unvaccinated against brucellosis. Other, fifteen animals (15) was apparently without clinical signs or history of abortion and was used as negative controls. Blood samples (10 ml) were drawn from jugular vein by using sterile disposable syringes, 5 ml and Sera were separated and transferred into sterile cryovials, storage at (-20°C) until testing by Rose Bengal test and another 10 ml were collected in heparinized vials for isolate lymphocytes.

Data of blood samples were summarized as infected group (25) and 15 buffalo as negative control group and the examined animals was categorized as under four years(≥ 4y) group including 25 buffaloes (18 infected and 7control) and over than 4 years (< 4y) group including 15 (7 infected and 8 control).

Rose Bengal Plate Test (RBPT):

All sera (40) had been tested using RBPT (Spineract, S.A.U) depending on standard procedure describe by Alton (Alton *et al.,* 1988).

Preparation and Isolation of blood lymphocytes:

Peripheral blood lymphocytes isolation according to (Nagahata *et al.,* 1994; Higuch and Nagaheta, 2000).

Comet assay (single cells gel-electrophoresis)

The comet assay was performed for detection the DNA damage in the buffalo peripheral blood lymphocytes using method Alkaline comet assay according to protocol described by (Peggy *et al.,* 2006).

Firstly the agarose slides were preparation by dipping slides in the normal molten (1.5% w/v) agarose which was preparation by mixing of the powdered agarose 0.75g and with (50ml) distilled water and that agarose was allowed for the air dry into thin film.

Red blood Cells suspension which prepared by added 5 ml of RBCs lysis buffer to each tube containing whole blood and after immediate gently vortex, these blood tubes were protected from light and put of incubator for 10-15 minutes, at room temperature then the cell suspension was centrifuge at (1500 rpm\2 min). The supernatant it is discarded and washed the pelletfor once with ice cold (PBS) (without Mg2+ and Ca2+) and centrifuged at 1500 rpm\2 min., the supernatant was discard, then , the cells sample was combined with low melting point agarose (was prepare by mixing 0.5g of agarose with (50ml) distilled water) at ratio 1:10 V/V and the mixture 75µl well immediately was add into comet slide by pipette. The slide was hold a horizontally then in to transferred to 4ºC in a cloudy container for 30 min., then the slides were transferred into a small basin containing pre chilled lysis buffer (2.5 M), (146.1gNaCl), (100 mM), (EDTA 37.2 g)., and (10 mM) Trise-base 1.2g ingredients were add to about (700 ml) distilled water then begin moving the mixture. The pH was adjust to (10.0) using 10 N NaOH and the volume was completed with distilled water to (890 ml), fresh 1% Triton (X-100), (10%) Dimethyl-sulfoxide (DMSO), and increase volume to the amount correct, and the slide was immersed in the buffer over-night (18-20h) at 4ºC in the cloudy place, after overnight, the slides were immersed with electrophoresis solution for 20min which prepared by dissolving 10.8g of 10 mM Trise-base and 0.93g of 100 mM EDTA in distilled water (900 ml). The pH was limit in to 13 (Alkaline pH) by use 10 N NaOH and the volume completed with distilled water to 1000 ml, then, slides were holding horizontally, and transferred to a horizontal electrophoresis chamber filled with a cold TBE electrophoresis solution, 24 volt (V) /cm and 300 (mA) was applied to the chamber for 18 min.

TBE electrophoresis solution was aspirated from chamber and replaced with Neutralization buffer (Tris-HC10.4M solution pH 7.5 for five min. to neutralize the cells.

50µl of diluted ethidium bromide dye was added to each well of comet assay slide and incubated at room temperature\15 minutes ,the slide was rinsed with distilled water for removestain excess and finally the slide was examined by fluorescence microscopy.

Three (3) criteria for measuring DNA damage were assessed to indicate that DNA parameter was tail length, (% DNA). in the tail and tail moment.

Statistical analysis

The obtained information was the statically analyze for mean and significant between the groups by using ANOVA in the SPSS program version -7.

Results

All infected buffaloes (25) were showed positive for

Rose Bengal test but the control group gave negative reaction ,while comet assay was revealed DNA damage in blood lymphocyte for all examined infected buffaloes is significant in comparison to control group , table 1.

Table 1: Detect DNA damage in lymphocytes of infected buffaloes by brucellosis and control by comet assay

Animal groups	Rose Bengal test			Comet assay test		
	Examined No.	Ve+	%	Examined No.	Ve+	%
Infected	25	25	100Aa	25	25	100Aa
Control	15	0	0Bb	15	0	0Bb

The different small and capital letters refers to significant variation at (p. ≤ 0.05)

In table 2 ,the assessment of DNA damage in lymphocytes as DNA tail % (48.75 ± 0.16), tail momentum (55.44 ± 0.25) and tail length (44.21 ± 0.05) in infected buffaloes were increased significantly than in control group as DNA tail % (1.6 ± 0.387), tail momentum (0.01 ± 0.003) and tail length (0.8 ± 0.1) respectively, Fig. 1.

Table 2: Results of comet assay in lymphocytes of control and infected buffaloes

Animal groups	Comet assay			
	Number	DNA tail %	Tail moment	Tail length
		M±SE	M±SE	M±SE
Infected	25	48.75±0.16A	55.44±0.25A	44.21±0.05A
Control	15	1.6±0.387B	0.01±0.003B	0.8±0.1B

The different capital letters refer to significant variations at (p≤0.05)

The effect of age of the infected buffaloes on damage of DNA lymphocytes was exhibited DNA breaks were increased with age(in buffaloes > 4 years) more than animals ≤ 4 years ,in which, values of (DNA) tail percentage, the tail length and tail momentum in old aged buffaloes was 28.63 ± 0.13, 31.81 ± 0.18, 25.08 ± 0.04 significantly more than values in younger buffaloes 20.12 ± 0.03, 23.63 ± 0.13, 19.13 ± 0.01 respectively. table 3.

These results indicate increased in the levels of (DNA) damage, by increasing the animal age, Fig. 2.

Discussion

This is the first study to detect DNA damage in lymphocytes of buffaloes infected with brucellosis by using of comet assay technique. Our results showed a statically significant change in all DNA measurements in peripheral blood lymphocytes in affected animals, in comparison with the control group suggest that the infection of lymphocytes

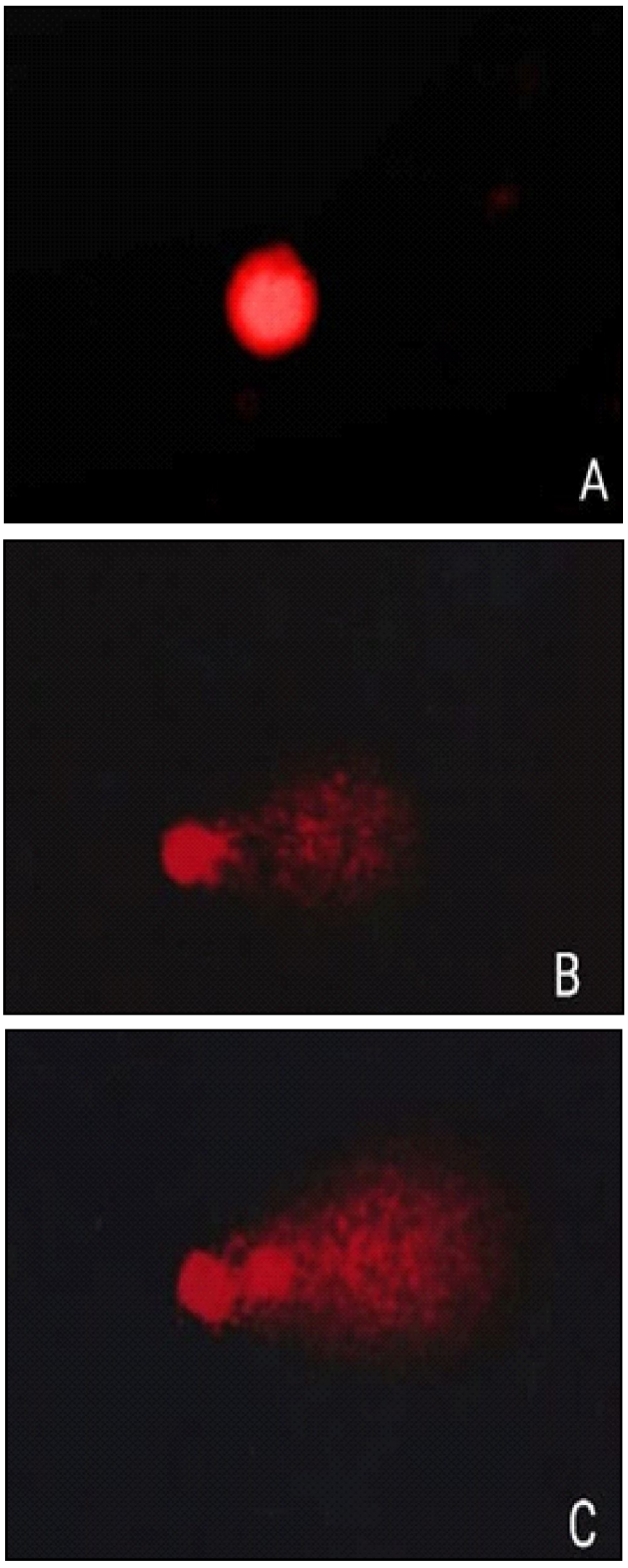
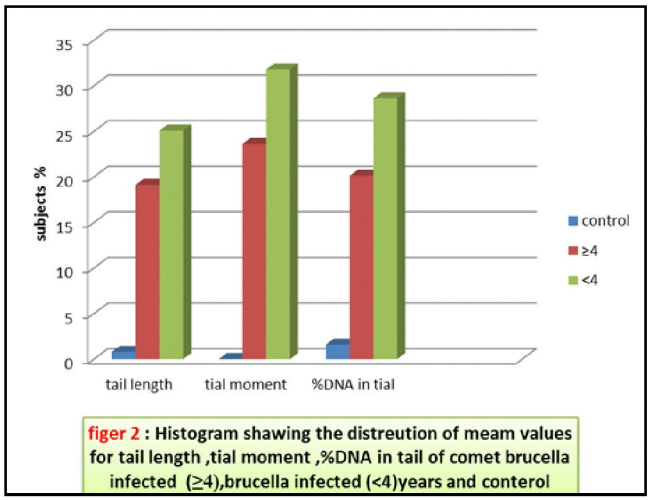


Fig. 1: Comet assay results in buffalo peripheral blood lymphocytes examined by florescent microscope (400X) of (A) the control group, shows fluorescent sphere without any DNA damage no tail., (B) Brucella infected group (≥4 years). And (C) Brucella infected group (<4 years) showsincrease heads fluore-scentaglow with tails this indicator to DNA damage. (Ethedium bromide stain).

Table 2: Gender of infected buffaloes affection on level of DNA damage

Age/ Years	Comet assay			
	Number	DNA tail % M±SE	Tail moment M±SE	Tail length M±SE
≥4	18	20.12±0.03B	23.63±0.13B	19.13±0.01B
Over than 4	7	28.63±0.13A	31.81±0.18A	25.08±0.04A



figer 2 : Histogram shawing the distreution of meam values for tail length ,tial moment ,%DNA in tail of comet brucella infected (≥4),brucella infected (<4)years and conterol

with brucellosis destroys the DNA and plays an important role as apoptosis-increasing factor that was in contract with (de Bagu¨e´s , *et al.,* 2004) who explained the ability of *brucella* spp. to produce cells toxicity by effect and varying plasma membrane and influence cell apoptosis, With (Elaine *et al*., 1996) and that The inflammatory responses caused by brucellosis produce oxidative agents. These agents are free radicals which destroy the nucleic acids of body cells. In brucellosis patient, there was a defective functions in T and NK lymphocytes. (Salmerón, *et al*., 1992; Rodriguez-Zapata *et al.,* 1996).

The results shows increase significant in the level of DNA damage by increasing animal age may be due to survival of *Brucella* spp. in body cell and the duration of the infection period and that in agreement with (Ramírez-Romero, 1998) whom described *Brucella* spp. as a facultative intracellular organisms which can invade with survive in cells for long period in the body and could establish lifelong. (Radhika, *et al*., 2012). These results demonstrated that the presence of brucellosis in buffalo was concerned to be common DNA damage in peripheral blood lymphocytes and it had been increased by increasing of infection period.

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