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Preliminarily Studies on Chromosomal Abnormalities and Sister Chromatid Exchanges Associated with Trypanosomosis in Relation to Male Camel Fertility

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Abstract: A total number 42 male camels in private farm, located at Ismailia province in Egypt during a period extended from 2004-2005, were examined by using parasitological and direct agglutination tests for diagnosis of trypanosomosis. The results revealed prevalence level 15(35.71%) and 33(78.57%) by parasitological and direct agglutination card tests respectively. Nine infected male camels with trypanosomosis were select to study chromosomal aberrations and Sister Chromatid Exchanges (SCE'S) frequency, as well as determined level of testosterone hormone. The frequencies of chromosomal structural aberrations in male camels with trypanosomosis were significantly increased 7.78±0.88 compared with non-infected control group 2.22±0.55. An increase in structural aberrations was observed in the form of fragment, deletions, gaps and breaks. In addition to, a significant increase in the frequency of SCE'S was observed more in diseased than in healthy camel. Thus, chromosomal abnormalities and SCE'S may be implicated in the pathogenesis of trypanosomosis.

Key words: Chromosomal aberrations, sister chromatid exchanges, trypanosomosis, male, camels

INTRODUCTION

The camel is usually used for a variety of purposes in arid and desert regions of the world. It is still an important animal production resource of meat, milk, hides and drought power (Tibary *et al*., 2005). Infections of the genital tract of camels may lead to temporary or permanent infertility either in male or female. Male infertility has been reported following severe systemic or local infections (Tibary *et al*., 2006).

Trypanosoma evansi is the most widely geographically distributed pathogenic trypanosome occurring in Africa, South and Central America and Asia (Luckins, 1988). It is a cosmopolitan parasite that transmitted mechanically by biting flies such as the tabanids and affects a wide range of hosts in which it may cause illness (Röttcher *et al*., 1987), including cattle, buffaloes, horses and camels, causing trypanosomosis, commonly known as surra (Claes *et al*., 2004).

In Africa, surra is most important in dromedary camels. The form of the disease may be acute, sub acute, chronic or inapparent (Wilson *et al*., 1983), but generally chronic form is the most common. In the acute form, trypanosomes are easily detectable in the blood and the disease is always fatal (Ventura *et al*., 2002; Claes *et al*., 2004). The disease is the most important single cause of economic losses in camel rearing areas, causing morbidity of up to 30.0% and mortality of around 3.0%

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(Pacholek *et al*., 2001). The losses in female were due to lower milk and meat yields, abortions, premature births and inability to feed the young (Artama *et al*., 1992). Chronically infected animals may survive for up to 3-4 years, causing heavy production losses in both male and female.

In male camels, trypanosomosis has been associated with severe testicular degeneration following bouts of fever and development of immune complexes that compromise sertoloi cell function (Omer *et al*., 1998; Al-Qarawi *et al*., 2004). The parasite has also been associated with impairment of pituitary function that may contribute to testicular degeneration and poor semen quality. Effects of trypanosomosis on testicular function disappear within 75-90 days after recovery of the male (Al-Qarawi *et al*., 2004; Kaufmann, 2005). However, infertility may persist beyond this period or become permanent in severe cases.

The diagnosis of disease is problematic because the commonly used tests have important limitations. Clinical signs are varied and non-specific while parasitological examination frequently fails to detect patent infection when parasitaemia is scanty in peripheral blood, especially in the chronic form (Chaudhary and Iqbal, 2000).

In field situations, rapid card agglutination tests may be more practical. Currently rapid tests are available, CATT/*T. evansi* for antibody detection, originally described by Bajyana Songa and Hamers (1988) and converted into a test kit by the Institute of Tropical Medicine, Belgium. High test accuracy has been reported for CATT/*T. evansi* in India (Pathak *et al*., 1997), Brazil (Franke *et al*., 1994), Mauritania (Dia *et al*., 1997) and Indonesia (Davison *et al*., 1999). In Mauritania, a large study reported an apparent prevalence of 15.6% using antibodies (card agglutination test-CATT/*T. evansi*) and 1.3% by parasite detection (Dia *et al*., 1997). In Sudan, a one-year survey showed an apparent prevalence of 31.3% based on antigen ELISA and 5.4% based on parasitological examination (Elamin *et al*., 1999). In Somalia, blood samples of 3000 camels were examined with parasitological techniques and 160 (5.3%) were found to be infected with *T. evansi* (Dirie *et al*., 1989). However, there are no published reports of the use of CATT/*T. evansi* in Egypt. Therefore, there is a need for trypanosomosis field-based diagnostic tests.

Alterations in chromosome number and structure are the best known of genetic based variations which have direct effects on fertility and reproductive outcome in animals (Berglund, 2001). Moreover chromosomal abnormalities were recorded associated with some infectious diseases as brucellosis in buffaloes and tuberculosis in cattle (Ghazy *et al*., 2007). However, it is also recorded as a cause of various infertility problems in both male and female animals (Mahmoud *et al*., 2005). The literature on trypanosomosis in relation to camel chromosomes is scarce. Therefore, in this study we evaluate the cytogenetic effect of trypanosomosis on Egyptian camel's lymphocyte. The formation of chromosomal aberrations and the induction of sister chromatid exchanges are the two cytogenetic parameters used in this study.

MATERIALS AND METHODS

Animals

A total number 42 male camels, aged over 5 years, in private farm, located at Salhia region at Ismailia province in Egypt during a period extended from 2004-2005, were examined for trypanosomosis. These animals were suffered from different symptoms, in the form of, sudden death, high body temperature, diarrhea and infertility. Nine infected male camels with trypanosomosis were selected to study chromosomal aberrations, as well as determined hormonal level and five apparently healthy animals were used as controls.

Serum and Whole Blood Samples

Blood samples were selected from all the examined animals by picture of the jugular vein into two sterile vacutainer tubes for each sample; one with heparin as anticoagulant and the other without. Blood

samples without anticoagulants were centrifuged in the laboratory at 2000 rpm for 20 min and sera were separated, labeled and stored at -20°C until analysed. Sera were examined for trypanosomosis by Card agglutination test. The whole blood samples from selected animals were used for parasitological and chromosomal analysis.

Diagnosis of Trypanosomosis in Male Camels

Parasitological Examinations

Parasitological diagnosis is carried out by the direct microscopic examination of blood (Luckins, 1992) and buffy coats (Murray *et al*., 1977).

Card Agglutination Test for Trypanosomosis (CATT/*T. evansi*)

This test is an experimental direct agglutination test for detection of antibodies in serum of infected animals. The antigen used consists of blood stream from trypanosomes RoTat 1.2, a variable surface Antigen type common to all *T. evansi* stocks examined hitherto. The test is done on a plastic card. Re-suspended antigen is mixed with diluted serum and agitated for 5 min. Blue clumping indicates a positive result (Davison *et al*., 1999).

Determination of Testosterone Hormone

Serum testosterone hormone level was determined using radioimmunological technique by using test kit obtained from Diagnostic Products Cooperation (DPC) Cataloge No. IKTTI., USA as described by Ismail (1986).

Chromosomal Aberrations and Sister Chromatid Exchanges

Blood samples were collected via sterile syringes from both infected and healthy camels. Each sample was divided into two halves, one for detection of chromosome aberrations and the other for Sister Chromatid Exchanges (SCE'S) frequency. Blood cells were cultured for 72 h at 38°C in 5 mL TCM-199, 1 mL fetal calf serum and 0.1 mL phytohaemagglutinin (PHA). After incubation, cells were treated with colchicines (0.05%) for 2 h, then with a hypotonic (0.075 M KCl) for 30 min. After fixation in acetic acid: ethanol (1:3) solution, the cells suspensions were dropped on wet slides then flamed to dry. The slides were stained with Giemsa stain and covered with DPX mounting media for chromosomal analysis. Chromosomal abnormalities were recorded in at least 50 metaphase spreads for each animal. For sister chromatid exchanges, 5-Bromodeoxyuridine (BrdU, Sigma) was added 48 h before harvesting. Then after hypotonic treatment and fixation, differential staining of sister chromatids was performed with fluorescence plus Giemsa method of Goto *et al*. (1978).

Statistical Analysis

Data were subjected to statistical analysis using t-test according to (Snedecor and Cochran, 1982).

RESULTS

Clinical Findings

At clinical examination, most of animals showed moderate signs of chronic form in the form of anemia, emaciation, recurrent fever, dullness, loss of condition, edema of the dependent parts and diarrhea. While, the remaining animals did not show any clinical evidence of the disease.

Diagnosis of Trypanosomosis in Male Dromedary Camels

Parasitological Examination Results

The microscopic examinations of blood and buffy coat film of camels revealed that 15(35.71%) out of 42 examined animals were positive for trypanosomosis (Table 1).

Table 1: Methods of diagnosis of trypanosomosis among male adult camels

	Microscopic examination		CATT/*T. evansi*	
No. of examined animals	No.	%	No.	%
42	15	35.71	33	78.57

Table 2: Chromosomal aberrations in camel lymphocytes infected with trypanosome

				No. of abnormal metaphases				Chromosome aberrations (Mean % ±SE)
Animals	No. of animals	No. of metaphases	No. of abnormal metaphases	Gaps	Fragment	Break	Deletion	without gaps
Control	5	250	16	6	4	4	2	2.22±0.55
Infected	9	450	53	18	20	8	7	7.78±0.88**

**: $p<0.01$ (t-test)

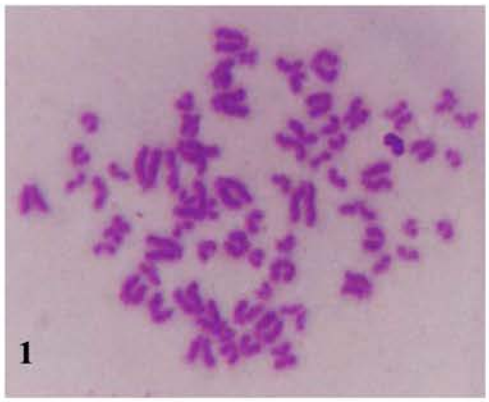

Fig. 1: Metaphases spread from blood cultured cells of non-infected camels showing normal metaphase

Serological Examination by Using Card Agglutination Test for Trypanosomosis (CATT)

Serological examination of camels serum using (CATT)/*T. evansi* test for detection of antibodies against *Trypanosoma evansi* revealed that 33(78.57%) were positive (Table 1).

Testosterone Hormone Profile

The results of serum level of total testosterone hormone in infected trypanosomosis and apparently healthy male camels revealed a significant decrease in the serum level of testosterone was 4.62±0.37 ng dL^{-1} compared with control camels 8.34±0.34.

Chromosomal Aberrations and Sister Chromatid Exchanges

The frequencies of chromosomal abnormalities increased significantly ($p<0.01$) in trypanosomosis infected camel (Table 2). The percentage reached 7.78±0.88 in diseased animals compared with 2.22±0.55 for the control. An increase in structural aberrations can be observed in the form of fragments, gaps, breaks and deletions (Fig. 1-3).

The frequency of SCE'S/cell in camel lymphocytes in relation to trypanosomosis represented in Table 3 and Fig. 4. The results showed a significant ($p<0.05$) increased in SCE'S frequency in diseased than control animals.

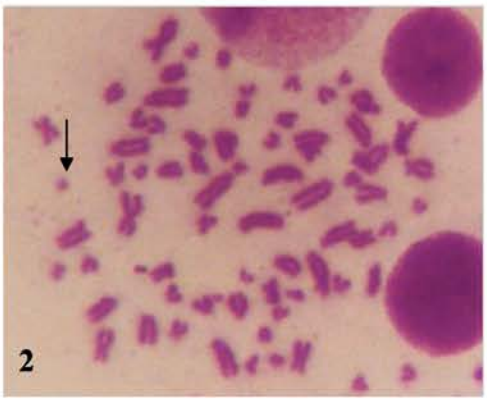

Fig. 2: Chromosomal aberrations in camels' lymphocytes infected with trypansomosis. Metaphases spread from blood cultured cells of infected male camels showing fragment

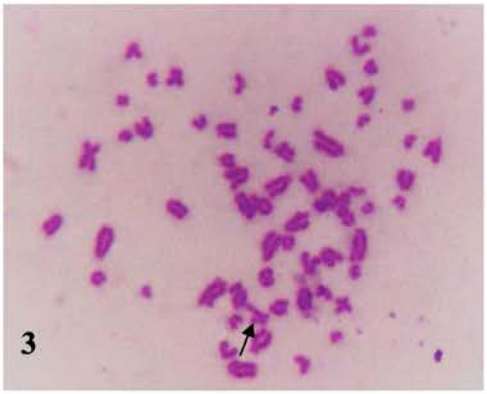

Fig. 3: Chromosomal aberrations in camels' lymphocytes infected with trypansomosis. Metaphases spread from blood cultured cells of infected male camels showing deletion

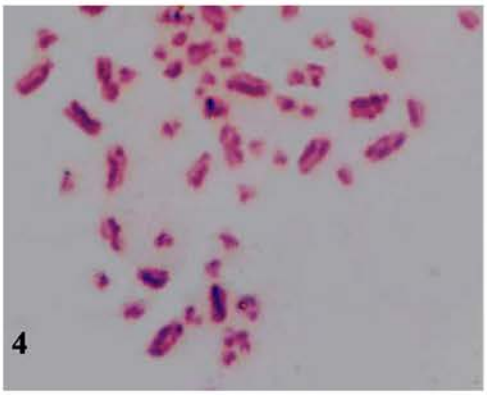

Fig. 4: Sister chromatid exchanges in camels' lymphocytes infected with trypansomosis

Table 3: Frequency of Sister Chromatid Exchange (SCE'S) in camel lymphocytes in relation to trypanosomosis

Animals	No. of animals	No. of metaphases with SCE'S	No. of SCE'S	SCE'S/cell Mean±SE
Control	5	125	401	3.21±0.45
Infected	9	225	1182	5.25±0.48*

*: $p<0.05$ (t-test), 25 metaphase/animal

DISCUSSION

Trypanosomosis in camels can occur in the acute or chronic form (Boid *et al*., 1986). The symptoms observed in these animals are characteristics of the chronic form, where, there are no pathognomonic signs of Surra and so laboratory diagnosis has to be carried out to confirm infection. Parasitological diagnosis is mainly carried out by the direct microscopic examination of blood and buffy coats. Present data demonstrate that, only 15 animals were positive with parasitological investigations from all examined animals. Present parasitological positive percentage results (35.71%) were higher than reported by Jacquiet *et al*. (1994) where 7.34% prevalence was seen in its survey. Also, Dia *et al*. (1997) observed only 1.4% positive by parasitological examinations. Meanwhile, Chaudhary and Iqbal (2000) observed less than 50% sensitivity by parasitological examinations. The wide range variation in parasitological results may attributed to regional characteristics, where from the history of purchased of these animals that coming from Haleeb and Shalateen (region near from north Sudan), where there were numerous animal watering points and the vector activities were capable of transmitting *T. evansi* abundant in this region as well as, parasitological examination frequently fails to detect patent infection when parasitaemia is scanty in peripheral blood, especially in the chronic form (Chaudhary and Iqbal, 2000). However, the test has a poor sensitivity, often less than 50% (Monzon *et al*., 1990; Nantulya, 1990; Luckins, 1992; Yadvendra *et al*., 1998). The implication of this is that in most situations *T. evansi* is under-diagnosed and the level of infection is greater than frequently reported.

During both acute and chronic *T. evansi* infections in camels pronounced immune response changes occur as increase in gamma-globulin (IgM) has been reported (Boid *et al*., 1981) but this is not protective. Present serological result was 78.57% by CATT/*T. evansi* test. The positive incidence was higher by this test due to its detection of specific antibodies. Present results agree with that reported by many authors whose coincides that CATT/*T. evansi* test is superior to parasitological examinations (Dia *et al*., 1997; Enwezor and Sackey, 2005).

The significant decrease in the serum level of testosterone infected trypanosomosis compared with control camels in this study may attribute to trypanosomosis may cause severe testicular degeneration. Moreover, the parasite has also been associated with impairment of pituitary function that may contribute to testicular degeneration and poor semen quality. Present results also explained by Omer *et al*. (1998) and Al-Qarawi *et al*. (2004) that trypanosomosis has been associated with severe testicular degeneration following bouts of fever and development of immune complexes that impairment sertoloi cell and pituitary function.

Present data demonstrate that, there is a significant increased in structural chromosomal aberrations and no increase in numerical aberrations in trypanosomosis camel. In this respect, chromosomal changes were also recorded in parasitic causing abortion as toxoplasmosis (Barakat *et al*., 2006). Moreover, several studies showed that clastogenic agents like certain viruses (Basrur *et al*., 1964; Hassanane *et al*., 1995; Sweify, 1999) lead to the chromosomal damage.

The other cytogenetic parameter studied in this work were sister chromatid exchanges. SCEs have been established as a cyto-diagnostic tool to assay the genetic damages induced by mutagens and carcinogens (Carrano *et al*., 1978; Perry, 1980; Rudek, 1985; Anderson *et al*., 1990). Moreover, SCE'S has high resolving statistical power and greater sensitivity than chromosome aberrations. Present

results showed that, a significant increase in the frequency of SCE'S was observed more in diseased than in healthy camel. These data coincide with the result of chromosome aberrations obtained in the present work. In addition to trypanosomosis, the increase frequency of SCE in this study may be due to camel age (Mahmoud *et al*., 2005).

CONCLUSION

Camel trypanosomosis is a disease of major economic importance. The serological test for diagnosis of trypanosomosis by using Card agglutination test (CATT/*T. evansi*) coincides that is superior to parasitological examinations. Also, trypanosomosis suggest that effect on male fertility in dromedary camels. Moreover, increased in structural chromosomal aberrations and no increase in numerical aberrations in trypanosomosis camel. In addition to, an increase in the frequency of SCE'S was observed in trypanosomosis camel. Thus, chromosomal abnormalities and SCE'S may be implicated in the pathogenesis of trypanosomosis. Therefore, genetic counseling and consideration of disease diagnosis should be an integral part of planning of control strategies.

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