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Preliminary Study on Chromosomal Aberrations Related to Brucellosis in Buffaloes and Bovine Tuberculosis in Dairy Cattle

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Abstract: A total number of 84 female buffaloes, located at Giza province in Egypt during a period extended from 2004 to 2005, were examined for brucellosis using different serological tests. Serological examination revealed prevalence level of 11.9% using RBPT and ARTK followed by TAT (9.5%), CFT (8.3%) and MET (7.1%). *Brucella melitensis* biovar-3 was isolated from tissue specimens collected from the brucella-positive seroreactive buffaloes during obligatory slaughter. Ten brucella-positive and ten brucella-negative reactors were selected for chromosomal analysis. The frequencies of chromosomal structural aberrations in buffaloes with brucellosis were significantly increased (5.40 ± 0.72) compared with non-infected control group (2.20 ± 0.45). An increase in structural aberrations was observed in the form of fragments, gaps, breaks and deletions. Moreover, a total of 50 dairy cows, collected from a small dairy farm exposed to an outbreak of Bovine Tuberculosis (TB) at Giza province in Egypt, were tested by tuberculin intradermal test using bovine Purified Protein Derivative (PPD) prepared from *Mycobacterium bovis* (*M. bovis*) and their corresponding milk samples were bacteriologically cultured for *M. bovis*. A total of 38/50 (76.0%) were positive reactors by single cervical test, while 25/50 (50.0%) were positive by caudal-fold test. Meanwhile, only 24/50 (48.0%) were tested positive by both of the two tests. *M. bovis* was isolated from milk samples of 19 out of the 50 examined cattle (38.0%). Nine tuberculin positive and nine tuberculin negative cattle were selected for chromosomal analysis. The frequencies of chromosomal abnormalities were also increased significantly in TB infected cattle (5.55 ± 0.52) compared with the control non-infected cows (3.11 ± 0.60). It could be concluded that structural chromosomal aberrations in the form of fragments, gaps, breaks and deletions were significantly increased in buffaloes with brucellosis and dairy cattle with bovine tuberculosis; which could be helpful on genetic control of these diseases.

Key words: Chromosomal aberrations, brucellosis, buffaloes, bovine tuberculosis, dairy cattle

INTRODUCTION

Brucellosis is economically considered as one of the most important diseases affecting farm animals and lead to great losses throughout abortion, stillbirths, reduction of milk yield and infertility (Refai, 2003). Besides the losses in animal wealth, brucellosis has a public health hazard; since man can be infected with *Brucella* spp. giving rise to undulant fever (Radostits *et al.*, 1994). Antibody detection is commonly used for diagnosis of brucellosis and in control programs. Several serological

tests that detect different classes and types of antibodies and vary in their sensitivity and specificity have been developed. Frequently, highly sensitive but less specific tests are used for screening purposes and are followed by more specific tests for confirmation purposes (Campbell *et al.*, 1994; Hirsh and Zee, 1999).

Bovine tuberculosis is a worldwide disease that causes a great harm on dairy farms and poses health risks to the population that consumes products of animal origin. It is still a problem with public health and economic importance in large areas of the world (Ritacco *et al.*, 1987). The economic losses caused by the disease are not only a reduction of 10-20% in milk production and weight, but also infertility and condemnation of meat. The loss is estimated to be 10-25% of the reproductive efficiency, excluding the losses from mortality (Lilenbaum *et al.*, 2001). The most common diagnostic assay for tuberculosis is tuberculin skin test. This test measures the delayed-type hypersensitivity response to an intradermal injection of purified protein derivative (Bates, 1996). Intradermal skin testing using PPD has proved to be an effective diagnostic test for identifying *M. bovis* infected cattle (Quinn *et al.*, 1994).

The commonly used test for genetic abnormalities is chromosomal analysis. Since chromosomal analysis provided critical information for diagnosis of pathological cases of human (Navsaria *et al.*, 1993) and animals (Mahmoud, 1997; Farag *et al.*, 1999), so it is recommended that a cytogenetic testing be performed on females intended for use as breeding stock. Dawson (1977) pointed out that abortions can be caused by hormonal imbalances, infectious agents or karyotypic abnormalities. Moreover, chromosomal abnormalities were recorded as a cause of early embryonic death (Mahmoud, 2001; Mahmoud *et al.*, 2002), abortion (Lin *et al.*, 1985; Causio *et al.*, 2002) and other infertility problems (Swartz and Vogt, 1983; Gustavsson, 1984; Mahmoud, 1997; Mahrous *et al.*, 2000; Mahmoud *et al.*, 2004). There are shortages in literatures about brucellosis and tuberculosis in relation to animal chromosomes. However, some researches have been undertaken to confirm genetic control of variation of resistance in cattle to brucellosis (Querish *et al.*, 1996; Adams and Templeton, 1998). These studies have demonstrated the potential of selection for brucellosis resistance in cattle to achieve rapid gains and thus have underscored the value of this important disease-resistant trait.

Therefore, the present study was undertaken mainly to study the chromosomal aberrations in buffaloes and dairy cattle in relation to brucellosis and bovine tuberculosis; which could be helpful on genetic control of these diseases.

MATERIALS AND METHODS

Animals

Buffaloes

A total number of 84 female buffaloes, located at Giza province in Egypt during a period extended from 2004 to 2005, were examined for brucellosis. They were suffering from different reproductive disorders in the form of abortions, repeat breeding and infertility. Ten brucella-negative and ten brucella-positive buffaloes were selected to study chromosomal aberrations (positive and negative reactors were confirmed by serological examination).

Dairy Cattle

A small dairy farm of Friesian cattle, located at Giza province in Egypt during 2005, was exposed to an outbreak of bovine TB associated with different reproductive disorders in the form of repeat breeding, endometritis, stillbirths, infertility, reduction in milk yield and mastitis. Out of which, a total number of 50 dairy cows were tested by tuberculin intradermal test (single cervical and caudal-fold tests) and their milk samples were cultured bacteriologically for TB. Nine tuberculin positive and nine

tuberculin negative (control) cattle were selected for chromosomal analysis (positive and negative reactors were confirmed by bacteriological examination of corresponding milk samples). These dairy cows proved negative seroreactors for brucellosis using different serological tests.

Serum and Whole Blood Samples

Blood samples were collected from all the examined animals by puncture 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 brucellosis by serological tests. The whole blood samples from selected animals were used for chromosomal analysis.

Serological Examination for Brucellosis

Rose Bengal Plate Test (RBPT), Tube Agglutination Test (TAT), Mercaptoethanol Test (MET) and Complement Fixation Test (CFT) were done as described by Alton *et al.* (1975 and 1988). All the used antigens were supplied by Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt. A titre of 1/40 (80 IU/mL) in TAT, 1/10 in MET and 1/8 in CFT or more is considered as brucella-positive reactor (Ruppanner *et al.*, 1980; Alton *et al.*, 1983). In addition, a commercial immunochromatographic ARTK assay (Antigen Rapid Brucella Ab test kit produced by Antigen Co., Kyunggi, Korea) was also used (Ghazi *et al.*, 2006).

Bacteriological Examination for *Brucella* spp.

Tissue specimens were collected, from 10 brucella-seropositive obligatory slaughtered buffaloes at Osem abattoir, under aseptic conditions for cultivation onto suitable selective *Brucella* agar media (Difco Laboratories, USA), incubated in 10% CO₂ incubator and examined after 10-14 days. The suspected colonies were identified according to Alton *et al.* (1988).

Tuberculin Intradermal Test

Bovine PPD tuberculin (2 mg mL⁻¹, 100,000 TU mL⁻¹), a purified protein derivative prepared from *M. bovis*, was supplied by Veterinary Serum and Vaccine Research Institute, Cairo, Egypt. Tuberculin is injected intradermally in the mid-cervical region (single cervical test) or in a skin-fold at the base of the tail (caudal-fold test) with a dose of 0.1 mL (10,000 tuberculin units). The reaction is read 72 h after injection and a firm swelling of 5 mm or more should be regarded as positive (Quinn *et al.*, 1994; Hirsh and Zee, 1999; Howard and Smith, 1999).

Bacteriological Examination of Milk for TB

Milk samples were collected from the selected cows under aseptic conditions for cultivation onto suitable media for *Mycobacterium bovis* which is Löwenstein-Jensen medium. The inoculated media was incubated at 37°C for up to 8 weeks. The identification of *M. bovis* was done in terms of microscopy (Ziel-Neelsen staining), cultural characteristics (enhanced growth with 0.4% sodium pyruvate, inhibited by glycerol) and biochemical reactions (urease+, nitrate reduction-, Niacin production-, inhibited by thiophen-2-carbonic acid hydrazide) (Quinn *et al.*, 1994).

Chromosomal Aberrations

The whole blood samples from selected animals were used for chromosomal analysis. Lymphocyte cultures were prepared according to Halnan (1977) with some modifications (Mahmoud, 1997). Blood cells were cultured for 72 h at 38°C in 5 mL tissue culture medium (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 suspension was dropped on wet slides then flamed to dry. The slides were stained with Giemsa stain and covered with Distyrene Plasticiser Xylene (DPX) mounting media for chromosomal analysis. Chromosomal abnormalities were recorded in at least 50 metaphase spreads for each animal.

Statistical Analysis

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

RESULTS

Serological Diagnosis of Brucellosis in Buffaloes

Serological examination of buffaloes with different reproductive disorders against brucellosis using different serological tests revealed prevalence level of 11.9% by RBPT and ARTK, followed by TAT (9.5%), CFT (8.3%) and MET (7.1%) (Table 1).

Bacteriological Examination of *Brucella* spp.

Brucella melitensis biovar-3 was isolated from tissue specimens of 10 previously examined brucella-seropositive buffaloes (positive by RBPT and ARTK) during obligatory slaughter at Osem abattoir.

Diagnosis of Bovine Tuberculosis in Dairy Cattle

Diagnosis of TB, in a small dairy farm exposed to a TB outbreak, was performed by single cervical and caudal-fold tuberculin intradermal tests using bovine PPD. A total of 38/50 (76.0%) were positive reactors by single cervical test, while 25/50 (50.0%) were positive by caudal-fold test. Only 24/50 (48.0%) were tested positive by both two tests (Table 2).

Bacteriological Examination of Milk for TB

It was found that 19 out of 50 examined cattle (38.0%) yielded *M. bovis* isolates from their milk samples (Table 2).

Table 1: Serological diagnosis of brucellosis among buffaloes with history of abortion and reproductive disorders using different serological tests

No. of examined animals	The different serological tests used for diagnosis of brucellosis				
	RBPT	TAT (positive at 80 IU mL ⁻¹ and higher)	MET (positive at 1:10 and higher)	CFT (Positive at 1:8 dilution)	Immunochromatographic Assay (ARTK)
84	No. 10 (11.9)	No. 8 (9.5)	No. 6 (7.1)	No. 7 (8.3)	No. 10 (11.9)

RBPT: Rose Bengal Plate Test, TAT: Tube Agglutination Test, MET: Mercaptoethanol Test, CFT: Complement Fixation Test, ARTK: Antigen Rapid Brucella Ab Test Kit, Values in parenthesis show percentage

Table 2: Diagnosis of bovine tuberculosis in dairy cattle exposed to an outbreak of TB using tuberculin skin tests and bacteriological examination of milk

No. of examined dairy cattle	Tuberculin intradermal test							Bacteriological examination of milk		
	Single cervical test		Caudal-fold test		Final results			Suspected	Positive	Negative
	Positive	Negative	Positive	Negative	Positive	Negative	Positive			
50	No. 38 (76.0)	No. 12 (24.0)	No. 25 (50.0)	No. 25 (50.0)	No. 24 (48.0)	No. 11 (22.0)	No. 15 (30.0)	No. 19 (38.0)	No. 31 (62.0)	

Values in parenthesis show percentage

Chromosomal Aberrations

Results in Table 3 showed a significant ($p < 0.01$) increase in percentages of structural chromosomal aberrations in buffaloes with brucellosis (5.40 ± 0.72) compared with the control group (2.20 ± 0.45). The frequencies of chromosomal abnormalities increased significantly ($p < 0.05$) in TB infected cattle (Table 4). The percentage reached 5.55 ± 0.52 in infected cattle compared with control non-infected cattle (3.11 ± 0.60). An increase in structural aberrations can be observed in the form of fragments, gaps, breaks and deletions (Fig. 1 and 2).

Table 3: Chromosomal aberrations in buffaloes lymphocytes infected with brucellosis

Animals	No. of animals	No. of metaphases	No. of abnormal metaphases	No. of abnormal metaphases				Chromosomal aberrations (Mean%±SE) without gaps
				Gaps	Fragment	Break	Deletion	
Control	10	500	18	7	6	3	2	2.20±0.45
Infected	10	500	38	11	14	8	5	5.40±0.72**

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

Table 4: Chromosomal aberrations in cattle lymphocytes infected with bovine tuberculosis

Animals	No. of animals	No. of metaphases	No. of abnormal metaphases	No. of abnormal metaphases				Chromosomal aberrations (Mean%±SE) without gaps
				Gaps	Fragment	Break	Deletion	
Control	9	450	21	7	4	6	4	3.11±0.60
Infected	9	450	36	11	10	9	6	5.55±0.52*

* $p < 0.05$ (t-test)

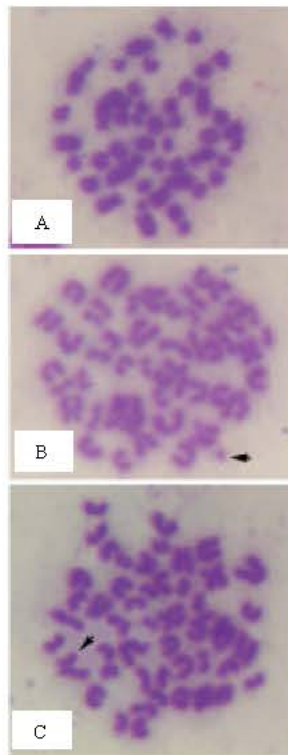


Fig. 1: Chromosomal aberrations in buffaloes lymphocytes infected with brucellosis, (A): Metaphases spread from blood cultured cells of non-infected buffaloes showing normal metaphase, (B): Metaphases spread from blood cultured cells of infected buffaloes showing fragment and (C): Metaphases spread from blood cultured cells of infected buffaloes showing deletion

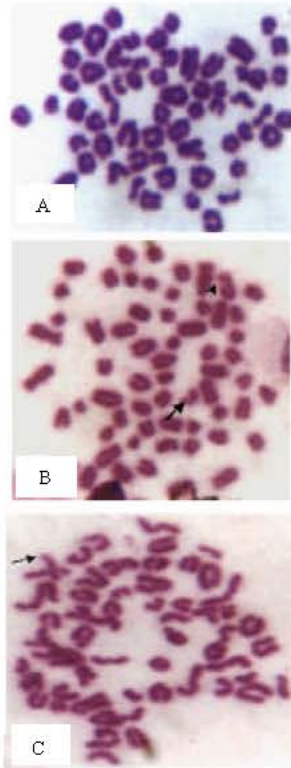


Fig. 2: Chromosomal aberrations in cattle lymphocytes infected with bovine tuberculosis, (A): Metaphases spread from blood cultured cells of non- infected cows showing normal metaphase, (B): Metaphases spread from blood cultured cells of infected cows showing break (short arrow) and fragment (long arrow) and (C): Metaphases spread from blood cultured cells of infected cows showing fragment

DISCUSSION

Brucellosis is a serious reproductive disease threatening the animal wealth and is of public health importance. The incidence of brucellosis in buffaloes with reproductive disorders in the current study revealed higher percent of positive reactors using RBPT and ARTK (11.9%) followed by TAT (9.5%), CFT (8.3%) and MET (7.1%). The incidence of brucellosis in buffaloes in Egypt was found to be lower (0.43-7.5%) by other authors (Abdel-Hafeez *et al.*, 2001; Ghazi *et al.*, 2006). The variations in incidence might be attributed to the difference in age, sex, breed, locality, management, stage of infection and immune status of animals (Tizard, 1987). The relatively high incidence in the current study might be attributed to the contact of buffaloes with other animals especially cattle. Results of this study cleared that infected buffaloes could be screened with a test such as RBPT or ARTK and confirmed with a more specific test such as CFT. Similar results obtained by Howard and Smith (1999), who reported that RBPT is a good screening test but should be supported by other serological tests. RBPT is still considered by several authors as more efficient, inexpensive and easily performed method in the detection of both early and chronic *Brucella* infection. RBPT detects IgM antibodies more efficiently than IgG₁ or IgG₂ antibodies and it was suggested that its acidic buffer inhibits immunologically the non-specific agglutinins (Allan *et al.*, 1976; Mikolon *et al.*, 1998).

Diagnosis of bovine tuberculosis is performed by the tuberculin intradermal test, which allows detection of cattle that have been exposed to *M. bovis*. Confirmation of diagnosis is made by isolation of *M. bovis* from milk samples. The tuberculin skin test is one test that correlates with a specific cell-mediated immune reaction. Animals infected with *Mycobacterium* bacteria develop characteristic delayed hypersensitivity reactions when exposed to purified derivatives of the organism. The reaction to injection is delayed because it takes a day or more for the T lymphocytes to migrate to the foreign antigen injected into the dermis (Pratt, 1997; WeiChong and XinAn, 2004). In positive cases, a swelling ≥ 5 mm develops within 72 h (Hirsh and Zee, 1999).

Present data demonstrate that, there was a significant increase in structural chromosomal aberrations and no increase in numerical aberrations observed in TB infected cattle. In this respect, an increased proportion of chromosomal aberrations were also observed in lymphocytes of tuberculous patients (Roman *et al.*, 1983; Rac *et al.*, 1991; Masjedi *et al.*, 2000). The frequencies of chromosomal structural aberrations in buffaloes with brucellosis were also significantly increased compared with the control group. On the contrary, Sikka *et al.* (1993) found no statistically differences between brucella-infected cows and control group. Several studies showed that clastogenic agents like certain viruses causing diseases; lymphosarcoma (Basur *et al.*, 1964), lumpy skin disease (Hassanane *et al.*, 1995) and hepatitis (Sweify, 1999) lead to chromosomal damage. Also, chromosomal aberrations were increased after vaccination against viruses (Gupta *et al.*, 1994; Genghini *et al.*, 2002). Moreover, chromosomal changes were also recorded in some parasitic diseases causing abortion as toxoplasmosis (Barakat *et al.*, 2006). Structural chromosomal aberrations accompanied by both diseases in this study were seen with metaphases containing more than one type of such aberrations in rare conditions and predominance of those with single aberration which explained the less adverse effect on the cell survival. In the same time, the rate of chromosomal aberrations was relatively low which is insufficient alone to cause reproductive disorders but may interact with other factors to increase the probability of reproductive diseases. In this context, environmental factors (Ahmed *et al.*, 1998) may play a role in the incidence of chromosomal abnormalities.

CONCLUSION

Results of this study concluded that brucella-infected buffaloes could be screened with RBPT and ARTK and confirmed with a more specific test as CFT. Moreover, single cervical tuberculin test together with caudal-fold test confirmed by bacteriological culture of milk could be helpful as diagnostic tools for bovine tuberculosis in dairy cattle. Structural chromosomal aberrations in the form of fragments, gaps, breaks and deletions were significantly increased in buffaloes with brucellosis and dairy cattle with bovine tuberculosis. Thus, chromosomal abnormalities may be implicated in the pathogenesis of brucellosis and bovine tuberculosis. Therefore, genetic counseling and consideration of disease diagnosis should be an integral part of planning of control strategies.

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