

## SOME EPIDEMIOLOGICAL, HAEMATOLOGICAL AND BIOCHEMICAL STUDIES ON BRUCELLOSIS IN CATTLE AND HUMAN CONTACTS IN SOME EGYPT GOVERNORATES

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### ABSTRACT

Received at: 29/9/2013

Accepted: 24/10/2013

For sero-biochemical study on brucellosis affected dairy cattle and human contacts for a period from June 2012 to May 2013, a total of 1210 blood samples were collected from dairy cattle in different localities in Dakahlia Governorate, 2378 blood samples in Damietta Governorate and 1600 blood samples in Alexandria Governorate. Another 120 blood samples from humans in contact with dairy cattle was performed. The collected sera were examined by Rose Bengal test (RBT), Buffered acidified plate antigen test (BAPAT) and Tube agglutination tests (TAT). The serological examination revealed that 216 (17.8%), 212 (8.9%) and 189 (11.8%) were positive in Dakahlia, Damietta and Alexandria Governorates respectively and also 20 samples humans contacts were positive. Haematological and biochemical examination of 7 positive samples collected from both infected cattle and diseased human compared with the healthy group revealed a significant ( $p < 0.01$ ) decrease in TRBCs, Hb concentration and PCV while TWBCs was significantly ( $p < 0.001$ ) increased. Biochemical estimation revealed a significant decreases in total proteins ( $p < 0.05$ ), albumin ( $p < 0.01$ ) and non significant decrease in total globulin. There was a significant ( $p < 0.01$ ), ( $p < 0.001$ ) increase in the activities of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) in cattle and human respectively. Analysis of some minerals in diseased cattle and humans showed a highly significant ( $p < 0.01$ ) decrease in calcium and inorganic phosphorus levels and a significant ( $p < 0.05$ ) decrease in magnesium, iron and sodium levels, besides non significant variations in the potassium level when compared with healthy one. It is thus recommended to the owners of the farms and yards must be hurry up for the early diagnosis of this serious zoonotic disease followed by the eradication of the sero-positive animals and vaccination of the negative animals. Moreover therapeutic plan should be cleared for the diseased farm workers. The cooperation between the Veterinary Services Centers and Public Health Authorities should be planed for the control of brucellosis in Egypt.

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**Key Words:** *Brucellosis, Epidemiological, Haemtological, Biochemical, Cattle, Human.*

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### INTRODUCTION

Brucella is a highly contagious zoonotic infectious organism. Infection is generally manifested clinically in animals by abortion; decrease of milk yield, breeding troubles and infertility while in human being a brucella infection cause undulant fever with its all complications, (Mehanna and Soliman, 1998). The estimated annual economic losses due to brucellosis were about 60 million Egyptian pounds yearly (AOAD, 1995). Because of being a serious zoonotic disease, brucellosis has been a subject of great concern of Official Veterinary Authorities and researchers since the disease was firstly reported by Bruce (1887). More than 70% of Egypt's total livestock population is owned by small holders, who keep a few cattle and

buffalo in their household as a source of milk and dairy products for home consumption or to sell, often unpasteurized, in local markets (El-Rafey, 2005 and Aidaros, 2005). The WHO considers brucellosis to be a neglected as a zoonotic disease, despite its widespread distribution and its effect on multiple species. It is not prioritized by national and international health systems, (WHO, 2005). Brucellosis was first reported in Egypt in 1939 and is now considered endemic in most parts of the country, (Refai, 2002). Despite its economic and public health importance, in recent years, the Official Egyptian Brucellosis Control Program does not appear to have been fully implemented, (Refai, 2002 and Hegazy *et al.*, 2009). Aggad (2003) recorded the disease in Egypt in Damietta (10.4%) and while in Dakahlia (7.18%) Governorates (El-Diasty, 2009). In 2007 a

population-based survey of acute febrile illness patients was conducted in Fayoum, a rural Governorate of Egypt, from the results of the survey human incidence of brucellosis was estimated to be 64 and 70 per 100 000 people in 2002 and 2003, respectively. If incidence estimates in the study had been based purely on hospital admissions, they would have been 3.8 per 100 000, (Jennings *et al.*, 2007). Brucellosis is considered an occupational hazard; risk factors identified for human brucellosis in the study were close contact with animals, exposure to aborted materials and consumption of dairy products. As close contact with animals is common in rural areas of Egypt and healthcare is not easily accessible for rural populations, rural residents are likely to have a high risk of brucellosis. The most effective way of reducing incidence in humans, is by controlling brucellosis in livestock, (Jelastopulus *et al.*, 2008 and Nicoletti, 1992).

Regarding the biochemical changes with brucellosis the available literature is little. The liver is the most important organ of the reticuloendothelial system (RES), that occupies a central position in the metabolism of the animal and the disturbance in hepatic function which may be accompanied with brucellosis in cattle have serious consequences for the productivity and reproductively of the diseased animals, (Deghedy *et al.*, 1990). Serological screening tests have been proved to be the most satisfactory monitor for brucella infection and during eradication programs, (Dokoo *et al.*, 1986). Successful control of the disease depends largely on successful diagnosis which in turn is based upon the use of efficient serological tests for picking up positive cases, (Hosein *et al.*, 2002). The object of this work was to study the sero and biochemical changes as a result of brucella infection in the diseased dairy cattle and human at high risk when compared with healthy ones.

## **MATERIALS and METHODS**

### **A) Prevalence of brucellosis:-**

#### **Animals and samples:**

A total of 5188 lactating cows; 1210 from Dakahlia Governorate, 2378 from Damietta Governorate and 1600 from Alexandria Governorate were examined in this study to determine the prevalence of brucellosis and studying biochemical changes as a result of brucella infection. Blood samples were collected from animals, human and sera separated.

#### **B) Antigens and tests used:**

Were obtained from Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt and used in the corresponding serological test for each.

#### **a- Buffered Acidified Plate Antigen Test (BAPAT):**

The test was carried out according to Alton *et al.* (1988).

#### **b- Rose-Bengal Plate Test (RBPT):**

The test was carried out according to Morgan *et al.* (1978).

#### **c- Tube Agglutination test (TAT):**

The test was carried out according to Alton *et al.* (1975)

#### **Signs on human cases:**

These cases suffer from undulant fever 39-41<sup>o</sup>c, sever back pain, heavy sweating with bad odour, influ-like symptoms, only one complicated case suffer from sever orchitis.

#### **Suggested treatment for human cases.**

The infected persons have been divided into two groups:

- First group take Rifamycin + Doxycyclin.
- Second group take Rifamycin + Doxycyclin + Amikacin.

#### **C) Biochemical and haematological examination:**

Blood samples of (7) positive cattle for brucellosis and (7) human cases of brucella infection were collected (part an EDTA as anticoagulated for haematological examination and the other part left for serum separation without anticoagulant for biochemical studies.

#### **Blood analysis:**

Hematological examination was performed according to Jain (2000). Serum total proteins according to Doumas, *et al.* (1981), albumin according to Frank (1950), AST, ALT activity levels according to Retman and Frankel (1957), while total globulin calculated mathematically by subtracting albumin from total protein. The calcium level was determined according to Gindler and King (1972), inorganic phosphorus El-Merzobani *et al.* (1977), magnesium Bauer (1982), iron Khan *et al.* (1995), sodium Guder *et al.* (1982), Potassium Henry (1974).

#### **Statistical analysis:**

All data were subjected to statistical analysis according to Snedecor and Cochran (1982).

## **RESULTS**

### **1- Prevalence of cattle brucellosis among individually reared animals in Dakahlia, Damietta and Alexandria Governorates.**

Results are shown in Table (1) and Figure (1).

Table 1: Prevalence of brucellosis among individually reared animals in Dakahlia, Damietta and Alexandria Governorates.

Governorate	Examined animals	Positive animals	Percent %
Dakahlia	1210	216	17.8
Damietta	2378	212	8.9
Alexandria	1600	189	11.8
Total	5188	617	11.9

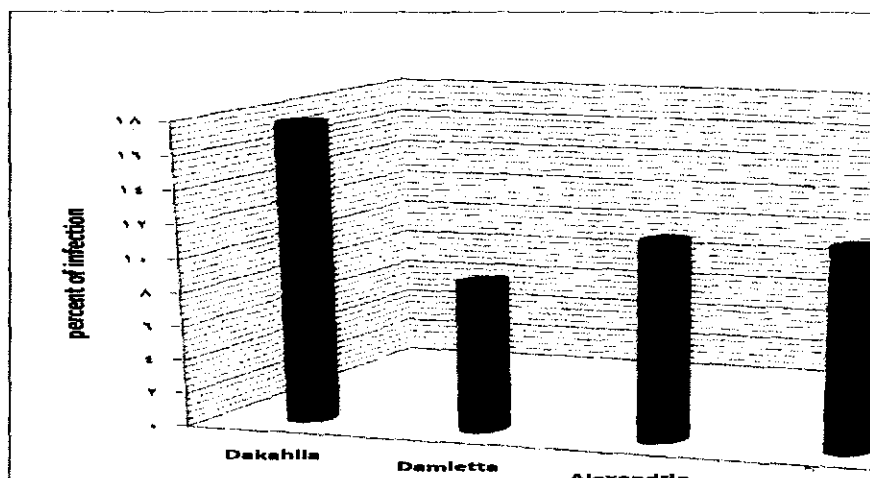


Figure (1) Prevalence of brucellosis among individually reared animals in Dakahlia, Damietta and Alexandria Governorates.

2 - Biochemical studies on the serum of (7) serologically positive cattle and human contacts:

Seven positive and (7) control cattle, (7) positive and (7) control human were used to determine mean values of some haematological parameters, mean values of liver function test and mean values of some macro and micro elements in examined dairy cows and human contacts. Results of haematological parameters are shown in Table (2), while biochemical parameters in Tables (3) and (4).

Table 2: Mean values of some hematological parameters in the examined dairy cows and human contacts.

Parameters	Cattle		Human	
	Control healthy	Serologically positive	Control healthy	Serologically positive
TRBCs ( $10^{10}/mm^3$ )	8.52±0.18	7.42±0.21** ↓	4.95±0.22	3.65±0.26** ↓
Hb (g/dl)	11.77±0.24	10.47±0.20** ↓	15.50±0.33	13.45±0.44** ↓
PCV (%)	33.41±0.88	28.30±1.34** ↓	44.20±1.81	36.80±2.18* ↓
TWBCs ( $10^{10}/mm^3$ )	9.80±0.25	12.95±0.46*** ↑	8.77±0.39	12.35±0.48*** ↑

\*Significant at  $P \leq 0.05$  \*\* Highly significant at  $P \leq 0.01$  \*\*\* Very highly significant at  $P \leq 0.001$

**Table 3:** Mean values of liver function test in the examined dairy cows and human contacts.

Parameters	Cattle		Human	
	Control healthy	Serologically positive	Control healthy	Serologically positive
Total proteins (g/dl)	7.46±0.14	6.58±0.27*	6.70±0.10	6.12±0.17*
Albumin (g/dl)	3.29±0.09	2.85±0.10**	4.39±0.18	3.09±0.25**
Total globulin(g/dl)	4.17±0.23	3.73±0.33 <sup>N.S.</sup>	2.31±0.26	3.03±0.31 <sup>N.S.</sup>
AST (U/L)	76.28±2.46	88.81±2.79**	8.11±0.22	15.25±0.32***
ALT (U/L)	24.55±1.38	32.80±1.46**	10.55±0.50	16.10±0.62***

\*Significant at  $P \leq 0.05$  \*\* Highly significant at  $P \leq 0.01$  \*\*\* Very highly significant at  $P \leq 0.001$   
 N.S. Non significant.

**Table (4):** Mean values of some macro and micro elements in the examined dairy cows and human contacts.

Parameters	Cattle		Human	
	Control healthy	Serologically positive	Control healthy	Serologically positive
Calcium (mg/dl)	11.95±0.42	9.25±0.47**	10.70±0.46	7.89±0.60**
Inorganic phosphoru (mg/dl)	6.74±0.29	5.19±0.30**	4.67±0.30	3.22±0.34**
Magnesium (mg/dl)	3.34±0.13	2.85±0.17*	2.35±0.15	1.83±0.17*
Iron (ug/dl)	223.50±2.06	215.13±2.46*	69.50±3.97	50.80±5.22*
Sodium (mEq/L)	140.12±2.06	132.90±2.40*	141.11±2.51	133.01±2.58*
Potassium (mEq/L)	4.99±0.14	4.73±0.13 <sup>N.S.</sup>	4.71±0.21	4.54±0.20 <sup>N.S.</sup>

\* Significant at  $P \leq 0.05$  \*\* Highly significant at  $P \leq 0.01$  N.S. Non significant

**Treatment:**

Co- treatment of these cases in co-operation with their doctors to coordinate the program of treatment. The twenty persons were five persons from different infected farms and 15 sporadic cases have been contacted with infected animals (cattle, buffaloes, sheep and goat) during parturition, abortion, retention of placenta and only one case during castration of infected buck suffer from orchitis.

The infected persons have been divided into two groups:

- First group treated by Rifamycin + Doxycyclin.
- Second group treated by Rifamycin + Doxycyclin + Amikacin.

**Dose:**

- Rifamycin 10 mg/ kg body weight per day every morning.
- Doxycyclin: 100mg / kg body weight twice a day.

**2- Symptoms in human and treatment:**

Twenty cases of human brucellosis were detected in present study. All the cases were serologically positive to BAPAT, RBPT and TAT. These cases have misdiagnosis from doctors as human typhoid but we suggest the infection with brucella due to the observed clinical signs positive serological tests, the relation between these cases and the veterinary field and the long term of wrong treatment.

These cases suffer from undulant fever 39-41° C, sever back pain, heavy sweating with bad odour, influ-like symptoms. Only one complicated case suffer from sever orchitis. Doctors have diagnosed this case as abscessiation of one tests and decide the castration, but after the right diagnosis as brucellosis, the case respond to the treatment after 4 days from the beginning of treatment.

buffaloes in Assiut Governorate), Khoudair and Sarfenaze (2007) (2.10% buffaloes in El-Bhaira, El-Menofia, El-Sharkia, Beni-Suef and Assiut Governorates) and (7.18% in Dakahlia Governorate and in Damietta Governorate 10.49%), El-Diasty and El-Beskawy (2012).

Such variation in the prevalence of brucellosis by different authors were attributed to variation in the sensitivity and specificity of the employed tests in each study, variation in localities and the rate of exposure in certain years.

#### **Haematological and Biochemical studies in examined dairy cattle and human contact:**

In present study (7) positive and (7) control cattle, (7) positive and (7) control human were analyzed for some haematological parameters, mean values of liver function tests and mean values of some macro and micro elements in examined dairy cows and human contacts. Regarding the serum biochemical changes, in the diseased dairy cattle and man with brucellosis, Table (2), revealed that both of total erythrocytic count (TRBCs), Hemoglobin concentration (Hb), and packed cell volume were highly significant decreased ( $p < 0.01$ ) when compared with the control group. The marked reduction could be attributed to the reduction of red cell formation as a result of inadequate production of erythropoietin hormone (Schalm *et al.*, 1986). These results completely agreed with those El-Olemy *et al.* (1984) and Ghazi *et al.* (2001). On contrary, the total leukocytic count (TLC) showed an opposite trend where the mean value of parameter very highly significant ( $p < 0.001$ ) increased over the level of the control group. The observed leukocytosis could be attributed to the activation of the lymphoreticular system for production and transportation of the antibodies in trial to combat the infection (Ahmed and Nada 1992). Recorded results were supported by those findings of Ghazi *et al.* (2001) and Ahmed *et al.* (2004) in camel who's recorded that leukocytosis might be due to stimulation of cell mediated immunity.

Concerning the biochemical profile results, (Table, 3) revealed significant and highly significant ( $p < 0.05$  and  $p < 0.01$ ) decrease in total proteins and albumin levels respectively and non significant increase in serum globulins in diseased men were estimated by comparing with the healthy one. Such results are similar to those recorded by Thabet *et al.* (1993) and Omima *et al.* (2003) who stated that brucella affected the concentration of the total protein and albumin due to hepatocellular failure to produce protein and albumin as a result of damaged liver cells. Hyperglobulinemia was attributed to humeral antibody response and increased proportion of the total titer representing. The activities of serum Aspartate Aminotransferase (AST) and Alanine

- Amikacin :7.5 mg / kg body weight twice a day I.M. for seven days

These two regimes of treatment and their doses were applied as recommended by the joint WHO (2005).

#### **Result of treatment:**

- 1 - The second group had a higher efficacy and more rapid action in terms of relief of symptoms compared to the first group, so adding Amikacin to first group in the treatment seems to be beneficial.
- 2- No adverse effect seen in the two groups.

## **DISCUSSION**

Blood serum samples of 1210 dairy cattle from Dakahlia Governorate, 2378 dairy cattle from Damietta Governorate and 1600 dairy cattle from Alexandria Governorate, Table (1), were tested for the prevalence of brucellosis. Different serological tests; namely Buffered Acidified Plate Antigen Test (BAPAT), Rose Bengal Plate Test (RBPT) and Tube Agglutination Test (TAT) were employed in this study for detection of brucella infected animals. An animal was classified as positive when two or more tests were positive.

The results summarized in Table (1) showed that 216 (17.8) of cattle in Dakahlia Governorate were positive reactors to brucella, while in Damietta Governorate, positive reactors were 212 (8.9%), but in Alexandria Governorate positive reactors were 189 (11.8%). The obtained results indicate the high prevalence of brucellosis among investigated cattle in these Governorates and the importance of the disease due to the expected losses as well as its public health significance.

These results also indicate that brucellosis is one of the important persistent and endemic diseases that have been reported at variable prevalences in Egypt. Prevalence of brucellosis has been determined by different authors at different localities as, Shalaby (1986) (in cattle 21.96%, 0.99%, 54.61%, 28.09%, 7.68% and 15.7% at El-Sharkia, El-Gharbia, El-Fayoum, El-Dakahlia, Alexandria and Giza Governorates), Hamouda (1989) (6.13% in cattle, 0.54% buffaloes and 8.5% sheep at El-Bhaira Governorates), Mahmoud (1989) (2.27% in cattle and 1.88% in buffaloes at Kafr El-Sheikh Governorate), Gadalla (1991) (1.75% cows and 1.88% buffaloes in Assiut Governorate), Abdel-Hafeez (1996) (0.89% cows in Assiut Governorate), Girgis (1998) (2.72% cows and 1.31% buffaloes in Beni-Suef Governorate), Youssif (1998) (1.36 cows, 0.39 buffaloes and 2.04% sheep in El-Menia Governorate), El-Diasty (2004) (4.95-5.8% cows in Dakahlia and Damietta Governorates), Nashwa and Afaf (2004) (8.57% cows in El-Kalubia Governorate), Bassiony *et al.* (2007) (4.29%-12.33% cow and 0.00%

In present study two regimens in treatment were tried, the first one was Rifampicin and Doxycycline and the second one was Doxycycline, Rifampicin and Amikacin. Better response was recorded in patients received the second regimen which included Amikacin. In spite of aminoglycosides have multiple side-effects such as nephrotoxicity or ototoxicity; no any of these side effects was recorded in the patients. This finding copes with Mitra *et al.* (2007) that had the same results in their studies. The authors concluded that, with short-term use of Amikacin for seven days, they did not have any aminoglycoside-specific side-effects (Mitra *et al.*, 2007).

The response of treatment with Doxycycline and Rifampicin was good, the recovery rate with the Doxycycline and Rifampicin regimen was 88% (Mitra *et al.*, 2007). In another study in Spain in 1995, the failure rate with the Doxycycline and Rifampicin regimen was 8% (Mitra *et al.*, 2007).

It is thus concluded that the use of Doxycycline and Rifampicin in treatment of patients with brucellosis had a good response. Adding amikacin for seven days in the treatment regimen will improve the results but the patients will need close follow up to avoid occurring of amikacin side effects.

It could be concluded that brucellosis plays a role in the remarkable disturbance of the biochemical parameters in the blood of the diseased cows and man. This reflects a disturbance in the general health condition of the host. More attention should be paid for careful, early diagnosis of this zoonosis followed by eradication of the sero-reactor dairy cattle, vaccination of non reactors and a therapeutic plan for the diseased farm workers. Veterinary authorities must encourage the cooperation between the Veterinary Services and Public Health Authorities to put a plan for the control of brucellosis in Egypt.

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Aminotransferase (ALT) enzymes as shown in (table, 3) showed a highly significant and very highly significant ( $p < 0.01$  and  $p < 0.001$ ) increase in diseased cattle and human contact respectively in comparison with the control group. The elevation in the enzymatic activities of the liver may be related to hepatic malfunction (Wattak and John 1971) as a result of increased breakdown of hepatocytes as in case of granulomates hepatitis (Benkirane 1997 and Young 1995). These results agreed with those findings of Ahmed *et al.* (2004) in camel and Khoudair and Sarfenaze (2007) in buffaloes. Concerning analysis of some minerals, Table (4) shows a highly significant ( $p < 0.01$ ) decrease in calcium and inorganic phosphorus levels below the control group level. The obtained results agreed with those of Coles (1986) and Tietz (1987) who attributed the hypocalcaemia and hypophosphatemia to non-diffusible albumin bound fraction caused by decreased protein associated with low albumin. It may be a result of decreased mobilization of calcium ions due to decreased metabolic activity during the disease condition, while Ahmed *et al.* (2004) reported non significant changes in camel brucellosis. It is evident from Table (4) that, there was significant ( $p < 0.05$ ) decrease in serum magnesium, and sodium levels, and non significant changes in potassium levels when compared with the control groups. This reduction may be due to anorexia and fluid lost during abortion and premature birth in animal (Hafez, 1980). This finding coincided with those of Nashwa and Afaf (2004). Significant ( $p < 0.05$ ) drop in the level of iron below the control groups was observed in positive cattle and human contact, as shown in Table (4). These results were completely agreed with those of El-Sawalhi *et al.* (1996) in camel and Ghazi, *et al.* (2001). The decreased level of serum iron may be attributed to the involvement of spleen in those animals in combating the infection and consequently disturbance of its function in storage and metabolism of iron, (Schalm *et al.*, 1986). Also decreased absorption of calcium and other minerals from the intestine were, due to diarrhea, vomition, resulting from severe colitis caused by brucellosis in man (Stermer *et al.*, 1991) is claimed.

### Treatment of human patients:

Brucellosis remains the most common zoonotic infection worldwide (Georgios *et al.*, 2005). The purpose of chemotherapy for brucellosis was to decrease symptoms, reduce complications, and prevent relapses (Aygen *et al.*, 2002). The World Health Organization (WHO) guidelines from (1986) are still considered the gold standard for the treatment of brucellosis, suggesting either the combination of Doxycycline and rifampicin for 6 weeks, or the combination of Doxycycline for 6 weeks with streptomycin for 2 or 3 weeks (Georgios *et al.*, 2005).

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بعض دراسات وبائية ودموية وكيميائية عن مرض البروسيللا في الأبقار والمخالطين الأدميين في بعض محافظات مصر

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أجريت هذه الدراسة للكشف السيرولوجي عن مرض البروسيللا في الأبقار الحلابة والمخالطين الأدميين بمحافظة الدقهلية ودمياط والإسكندرية وذلك في الفترة من يونيو ٢٠١٢ حتى مايو ٢٠١٣ وكذلك تم فحص بعض العينات الموجبة للتعرف على التغيرات الدموية والبيوكيميائية التي قد تحدث نتيجة الإصابة بميكروب البروسيللا. ولهذا الغرض تم تجميع عدد ١٢١٠ عينة دم من الأبقار الحلابة بمناطق مختلفة بمحافظة الدقهلية ، ٢٣٧٨ عينة بمحافظة دمياط ، ١٦٠٠ عينة بمحافظة الإسكندرية وكذلك تم جمع ١٢٠ من عينات دم من العاملين بهذه المزارع وتم فحص العينات سيرولوجيا باستخدام إختبارات الـ روزبنجال ، الأنتجين الشريحي المحمص والتلازن الأنوبي البطي وأسفرت النتائج أن ٢١٦ عينة (١٧.٨%) بمحافظة الدقهلية ، ٢١٢ عينة (٨.٩%) بمحافظة دمياط ، ١٨٩ عينة (١١.٨%) بمحافظة الإسكندرية و ٢٠ عينة من العاملين كانت موجبة للاختبار. وبالنسبة للقياسات الدموية والبيوكيميائية الحيوية في سبعة من العينات الموجبة للاختبارات السيرولوجية في كلا من الماشية والإنسان. أسفر الفحص الدموي عن نقص شديد في عدد الكلى لكرات الدم الحمراء وتركيز الهيموجلوبين وحجم الخلايا المصمتة ووجد أيضا زيادة معنوية كبيرة في العدد الكلى لكرات الدم البيضاء مقارنة بالمجموعة الضابطة. وأسفر الفحص الكيميائي عن وجود نقص معنوية في مستوى البروتين الكلى والألبومين. ولوحظ ان هناك زيادة معنوية في النشاط الإنزيمي للكبد وهي إنزيم الألتين أمينوترانسفيريز (ALT) وإنزيم الأسبرتيت أمينوترانس فيريز (AST). كما وجد نقص معنوي في مستوى الكالسيوم والفسفور الغير عضوى والماغنسيوم والحديد والصوديوم في حين وجد نقص غير معنوي في مستوى البوتاسيوم. هذا وقد تم توصية أصحاب المزارع والحظائر بالتخلص من الأبقار المصابة بالبروسيللا وتحصين الأبقار السليمة وعلاج العاملين المصابين تحت الإشراف الطبي. كما ننصح بالتعاون بين كل من الهيئة العامة للخدمات البيطرية ومنظمة الصحة العالمية لوضع خطة لمكافحة والقضاء علي مرض البروسيللا في مصر.