

PREVALENCE STUDY ON BRUCELLOSIS IN SOME RUMINANTS SLAUGHTERED OUT OF ABATTOIRS IN ASSIUT GOVERNORATE

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ABSTRACT

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In this study, 482 serum and tissues samples including spleen and lymph nodes were collected from cattle (215), buffaloes (103), sheep (105) and goats (59), which had been slaughtered out of abattoirs in different localities in Assiut Governorate. The serum samples were examined for the detection of antibodies against *Brucella* spp. The results of screening tests Buffer acidified plate antigen test (BAPAT) and Rose Bengal plate test (RBPT) gave 36 seroreactive animals by incidence of 10.23% in cattle, 2.91% in buffaloes, 7.61% in sheep and 5.08% in goats, respectively. All positive serum samples were further retested by Standard serum agglutination test (SAT), Rivanol test (RIVT) and indirect enzyme linked immunosorbent assay (ELISA) as confirmatory tests. SAT gave 90.9% in cattle and 100% in buffaloes while RIVT gave 86.3% in cattle and 100% in goats of seroreactive animals. Moreover, ELISA gave 95.4% in cattle, 100% in buffaloes, 87.5% in sheep and 100% in goats of the seroreactive animals. Eleven isolates (30.3%) of *brucella melitensis* biovar 3 were recovered from 36 seroreactive animals. These isolates represent 6 (27.3%) for cattle, 1 (33.3%) for buffaloes, 3(37.5%) for sheep and 1(33.3%) for goats. In conclusion, *Brucella melitensis* was wide spread in ruminants slaughtered out of abattoirs in Assiut Governorate which cause a serious infection in human and animals.

Key words: Prevalence - brucellosis – ruminants- slaughtered -out - abattoirs - Assiut

INTRODUCTION

Brucellosis is an important re-emerging zoonosis with worldwide distribution. It stills an uncontrolled serious public health problem in many developing countries including Egypt (Mantur and Amarnath, 2008 and Samaha *et al.*, 2009). Although brucellosis has been controlled in most industrialized countries, it remains a major problem in the Mediterranean region, western Asia, Africa, and Latin America (Pappas *et al.*, 2006). Brucellosis causes appreciable economic losses in livestock industry because of abortions, retained placenta, decreased milk production, sterility in males due to orchitis and veterinary care in cattle, sheep and goat as well as treatment costs in human (Corbel 1997 and Adams, 2002). Unfortunately, the applied control measures may not be capable of reducing the levels of infection in ruminants (Hegazy *et al.*, 2009). Brucellosis for several decades has been recognized as a significant public health problem which includes chills, fever, malaise and headache, requiring prolonged treatment (Korman, 1988). In the Middle East, Benkirane (2006) suggested that its incidence is increasing in ruminants and humans.

Brucella melitensis biovar 3 is considered to be the predominant species of brucella isolated from humans and animals in Egypt (Refai, 2002). Outbreaks in cattle due to *B. melitensis* have become a worldwide emerging problem particularly difficult to control due to lack of knowledge on the epidemiology in this host species and of ineffective vaccination (Alvares *et al.*, 2011). Prevalence of brucellosis in cattle and buffaloes based on a survey studies published between 1948 and 2009 in Egypt nearly was 5.4 % by BPAT (Gwida *et al.*, 2010). The study by Kaoud *et al.* (2010) revealed that prevalence of *Brucella* among herds of cattle, sheep and goats in certain Governorates using RBPT were 21.6%, 26.6% and 18.8%, respectively. When RBPT positive samples were subjected to ELISA test, the percentages were 17.2%, 21.2% and 14.5%, respectively. Moreover, in a recent study the incidence of brucellosis was 8 % in cattle, 1 % in buffaloes and 4 % in sheep, Horton *et al.* (2014). Incidence of brucellosis in Assiut Governorate was ranged between 0.57 % to 1.34% in cattle by Abedel-Hafeez (1996) and Koriem *et al.* (2013), in buffalo 3.03%, to 3.35 %by Samaha *et al.* (2008) and Koriem *et al.* (2013), in sheep 1.1% to 1.35% by Sedeek (1999) and Abedel-Hafeez *et al.* (2001) and finally in goats the incidence ranged

between 0.27% to 0.94% by Nashed (1977) and Mohammed (2001).

Bacterial load in animal muscle tissues is low but consumption of under cooked traditional delicacies such as liver has been implicated in human infection (Tikare *et al.*, 2008). The transmission of brucella infection and its prevalence in a region depends upon several factors like food habits and methods of processing (Mantur *et al.*, 1996). Unsafe butchering is considered to be one of the major risk factors for human infection with *Brucella species*. The organism could be detected in swabs collected from butchers hands, knives, tables and meat displayed for sale (Uche and Agbo, 1985). Those with a professional risk of acquiring infection include livestock producers, abattoir workers, shepherds, farmers, and veterinarians. The much higher seroprevalence rate has been also noted in abattoir workers (Barbuddhe *et al.*, 2000, Mantur and Amarnath, 2008). Unhygienic disposal of slaughtered animals parts i.e. blood, tissues, gravid uterus, infected fluid, fetal membranes as well as manure interferes with brucella controlling programs (Ramos *et al.*, 2008).

It was found that BAPAT and RBPT serological tests gave the highest rate of sensitivity that guides us to use these tests as screening tests on animals brucellosis. RIVT. showing the highest rate of specificity that bearing in mind the BAPAT and RBPT positive samples should be confirmed by this test, Montasser *et al.* (2011). ELISA has been reported as a very sensitive and specific test for the diagnosis of brucellosis (Raúl *et al.*, 2005). The gold standard that confirms the presence of the diseases is the isolation, identification and biotyping of the

bacterial agent (Cunningham (1977) and Alton *et al.*, 1988). On the other hand Hamdy (1997) revealed that the definitive diagnosis for brucellosis requires the recovery of the organism but it is difficult due to the fastidious nature of organism and in case of mild infection. Therefore, diagnosis has been based mostly on the results of serological tests. For brucellosis the Serological tests detect the presence of anti-lipo-polysaccharide (anti- LPS) antibodies, measure total antibodies or measure the level of different immunoglobulins (Wright and Nielsen 1990). Usually a battery of tests is used for serologic testing of brucellosis as there is no single test capable for detecting all infected animals (Ibrahim *et al.*, 1999). Tests currently used for the serological diagnosis of infections in sheep and goats were initially developed the same for the diagnosis of infections in cattle (SANCO 2001).

The present work aimed to document the frequency of brucellosis among different ruminants slaughtered out of abattoirs in Assiut Governorate through performing screening and confirmatory serological tests and isolation of brucella organism from tissues of the slaughtered animals.

MATERIALS and METHODES

1- Collection of samples:

Serum and tissue samples (lymph nodes and spleen) were aseptically collected from (482) Egyptian native breed animal species which slaughtered out of abattoirs in different localities at Assiut Governorate as explained in Table (1). These samples were collected during the period from January 2013 to July 2014.

Table 1: Samples distribution from different localities in Assiut Governorate and their sex of slaughtered food animals.

Animal species	No. of samples	Locality		Sex	
		North Assiut	South Assiut	Male	Female
Cattle	215	150	65	0	215
Buffaloes	103	18	85	5	98
Sheep	105	30	75	12	93
Goats	59	24	35	8	51
Total	482	222	260	25	457

2- Serological tests:

The collected serum samples (482) were examined by BAPAT and RBPT as screening tests. Then all positive serum samples were further retested by SAT, RIV T. and ELISA as quantitative confirmatory tests. The antigens of BAPAT, RBPT, SAT and RIVT. were supplied by Veterinary Serum and Vaccine Research Institute – Abbasia, Cairo- Egypt and performed according to Alton *et al.* (1988). ELISA antigen was supplied from Synbiotics Europe 2, rue A

– Fleming 69007 Lyon –France. Serum samples was performed by ELISA as mentioned by Jimenez *et al.* (1992).

3- Bacteriological examination:

Supramammary and inguinal lymph nodes and parts from spleen were collected from 482 animal species at the time of slaughter. Samples were directly taken to the laboratory in ice-box and kept in deep freezing (-20°C) until serological tests were performed. Tissue

samples from the seropositive animals (36) were examined bacteriologically according to (Alton *et al.*, 1975 and Magwedere, 2011). Tissue samples were thawed over night at 5°C. The lymph node was sliced into half exposing the inner surface, which was minced for 2 to 3 min. with a sterile scalpel. The content of lymph node was mixed with an equal volume of isotonic phosphate-buffered saline (pH 6.3) and blended in a laboratory blender for 5 min. Fifty grams of spleen was mixed with 100 mL of isotonic phosphate-buffered saline (pH 6.3) and blended in a laboratory blender for 5 min. The macerated tissue suspension was spread with a sterile cotton swab over the entire surface of agar plates of Farrell's medium blood agar base (oxid)

supplemented with 10% equine serum, 1% glucose and brucella selective supplement (Oxoid). The plates were incubated at 37°C in an aerobic atmosphere containing 10% CO₂. Cultured plates were examined for growth on day 4 and daily for 4 weeks. Suspected colonies were subcultured on Brucella agar slants (Oxoid) for further identification. The isolates were identified according to morphologic characteristics, microscopic appearance, slide agglutination tests with (anti-S brucella serum, anti-R brucella serum), Lactose fermentation, growth on MacConkey agar; Haemolysis on blood agar, Motility at 37°C, Oxidase, Urease, Nitrate reaction, sensitivity to dyes and citrate utilization.

RESULTS

The obtained results were recorded in tables 2- 5

Table 2: Seroprevalence of brucellosis in different animal species using screening tests (BAPAT and RBPT).

Animal species	No. of samples	Screening tests			
		RBPT		BABAT	
		+Ve	%	+Ve	%
Cattle	215	22	10.23	21	9.76
Buffaloes	103	3	2.91	3	2.91
Sheep	105	8	7.61	8	7.61
Goats	59	3	5.08	3	7.61
Total	482	36	7.46	35	7.26

Table 3: Seroprevalence of brucellosis in positive reactor animals detected by confirmatory tests.

Animal species	No. of samples	Screening tests				Confirmatory tests					
		BAPAT		RBPT		SAT		RIVT		ELISA	
		+Ve	%	+Ve	%	+Ve	%	+Ve	%	+Ve	%
Cattle	22	22	100	21	95.4	20	90.9	19	86.3	21	95.4
Buffaloes	3	3	100	3	100	3	100	2	66.6	3	100
Sheep	8	8	100	8	100	6	75	5	62.5	7	87.5
Goats	3	3	100	3	100	2	66.6	3	100	3	100
Total	36	36	100	35	97.2	31	86.1	29	80.5	34	94.4

Table 4: Seroprevalence of brucellosis in different animal species in relation to their sex and locality based on ELISA.

Animal species	Total	Locality						Sex					
		North Assiut			South Assiut			Female animals			Male animals		
		No. of samples	+Ve	%	No. of samples	+Ve	%	No. of samples	+Ve	%	No. of samples	+Ve	%
Cattle	215	150	18	12.0	65	3	4.61	0	0	0	215	21	9.76
Buffaloes	103	18	1	5.5	85	2	2.35	5	0	0	98	3	3.06
Sheep	105	30	2	6.6	75	5	6.66	12	1	8.3	93	6	6.45
Goats	59	24	1	4.1	35	2	5.7	8	0	0	51	3	5.8
Total	482	222	22	9.9	260	12	4.6	25	1	4	457	33	7.2

Table 5: Prevalence of brucella organism in seroreactive animals.

Animal species	Total serologically positive	Total bacteriologically positive	
		No.	%
Cattle	22	6	27.3
Buffaloes	3	1	33.3
Sheep	8	3	37.5
Goat	3	1	33.3
Total	36	11	30.3

DISCUSSION

The principal objective of using serological tests in control and eradication programs of brucellosis is to detect infected animals that may cause spread of the disease. Unfortunately, there is no single test can identify all infected animals at all stages of the disease, therefore a combination of serological tests should be included to reduce the number of both false negative and false positive serological reactions (Cordes and Carter, 1979).

In the present study, Table (2) shows the incidence of brucellosis among slaughtered animals out of abattoirs in Assiut Governorate. By using screening tests BAPAT and RBPT the incidence of brucellosis were 10.23%, 9.76%, 2.91%, 2.91%, 7.61%, 7.61%, 5.08% and 5.08% in cattle, buffalo, sheep, and goats, respectively. This result was nearly similar to that reported by Montasser *et al.* (2001) who recorded that the incidence among cattle was 7.5%, 10%, 7.75%, 9%, 7.12% and 7.12% by using CFT, BAPAT, RBPT, SAT, MET and RIVT, respectively. On the other hand, this result was much higher than that recoded by Koriem *et al.* (2013) who found that the incidence of Brucella in cattle slaughtered in governmental abattoirs was 1.34%. On contrast our

result is lower than that recorded by Kaoud *et al.* (2010), Holt *et al.* (2011) and Horton *et al.* (2014).

Regarding buffaloes results revealed that the prevalence was 2.91% by both BAPAT and RBPT. The obtained result was nearly similar to that recorded by Samaha *et al.* (2008) but lower than that reported by Ali and Mahdey (2010), Holt *et al.* (2011) and daSilva *et al.* (2014) who cited the infection rate ranged between 7.6 % 15.5% and 12.4 %. The obtained results revealed that the incidence of infection was higher in cattle than in buffalo. This may be due to the fact that buffaloes have more resistant to the disease than cattle (Fosgate *et al.*, 2011).

Concerning sheep the prevalence of brucellosis in the present study was 7.61% by BAPAT and RBPT. This result was much higher than those cited by Sedeek (1999) and Abdel-Hafeez *et al.* (2001) but lower than that reported by Hegazy *et al.* (2011). Our results in goats showed that the prevalence of brucellosis was 5.08 % by BAPAT and RBPT. This was nearly the same which was reported by Montasser *et al.* (2011), but much higher than Nashed (1977) and Abd-El-Kader (1996) who pointed that the incidence was 0.82 % and 0.33 % using RBPT screening test. Moreover, high prevalence rate was noticed in sheep and goat by

Kaoud *et al.* (2010). The high ratio of brucellosis in sheep and goats may be due to free grazing and movement of these flocks which contribute to the wide distribution of brucellosis in these animals and to other animal species (Mantur and Amarnath 2008).

In Table (3) the results indicated that BAPAT gave of 100% in all tested seroreactor animals while RBPT gave 95.4% in cattle. This finding was in agreement with Angus and Barton (1984) and Montasser *et al.* (2012). In addition to Gall and Nielsen (2004) who mentioned that BAPAT was more sensitive and accurate than other conventional tests for detection of brucella in bovine serum. This may be due to the instability of some antigen preparations used in the other tests as RBPT which may deteriorate when repeatedly cycled between refrigerator and room temperature during use (MacMillan, 1990).

The SAT gave of 90.9% in cattle and 100% in buffaloes while RIVT gave 86.3% in cattle and 100% in goats of the seroreactive animals. Moreover, ELISA gave 95.4% in cattle, 100% in buffaloes, 87.5% in sheep and 100% in goats of the seroreactive animals.

The overall percentage of seropositive reactors detected by BAPAT were (100%), RBPT (97.2%), SAT (86.1%), RIVT. (80.05%) and ELISA (94.4%). These results were in agreement with the finding of Abdoel and Smit (2007) and Montasser *et al.* (2012). The radical change in the incidence of the serological tests between screening tests (BAPAT and RBPT) and confirmatory tests (SAT, RIV.T. and ELISA tests) was due to the activity of specific and non-specific antibodies (Alton *et al.*, 1988). Our finding revealed that RIVT. and ELISA are good confirmatory tests for diagnosis of caprine brucellosis this also reported by Mohammed (2001). Moreover, ELISA consider an excellent test for diagnosis of brucellosis in ruminants which has high sensitivity and specificity which agree with the finding of Raúl *et al.* (2005) and Montasser *et al.* (2012).

The obtained results in Table (4) showed that cattle and buffalo which slaughtered at north Assiut had more incidence of brucellosis than that of south Assiut. This result was coincides with that obtained by Montasser *et al.* (2011) and Koriem *et al.* (2013). Moreover, in Table (4) showed a high incidence in female animals than that of male animals. Similar result was recorded by Isloor *et al.* (1998) and Junaidu *et al.* (2011). The high ratio in seroreactive female animals in the present study may be due to most of these animals were female population, senile with a history of reproductive disorders and or emaciated as a mainly cause for culling. In the same manner Kazi *et al.* (2005) noted that the antibody titer against Brucella appears to be associated with the age as young female animals may be harbor the organism

without expressing any detectable antibody till first parturition or abortion.

Taking into consideration that it is not always possible to recover brucella organisms from all organs of all infected animals also negative bacteriological investigation dose not exclude the presence of brucellosis as pointed out by Robertson *et al.* (1977). In Table (5) 11 isolates of *brucella melitensis* biovar 3 were recovered from 36 seroreactive animals from their lymph nodes and spleen. These isolates represent 6 (27.3%) for cattle, 1 (33.3%) for buffaloes, 3 (37.5%) for sheep and 1 (33.3%) for goats. Moreover, the overall mean isolation rate was 30.5 % in relation to the seroreactive animals. Our results nearly similar to Esmail *et al.* (2008) and Al-Farwachi *et al.* (2010) who isolated 4 out of 12 seropositive samples (33.3%). On the other hand, our results is higher than that reported by Salem and Hosein (1990) but was lower than that cited by Ali and Mahdey (2010) and Montasser *et al.* (2011) who their isolation rate reached to 50% and 38.3%, respectively from spleen lymph nodes.

CONCLUSION

Brucella melitensis was wide spread in ruminants slaughtered out of abattoirs in Assiut Governorate. The absence of pre and post mortem examination and unhygienic disposal of blood, genital organs and infected lesions in out abattoir slaughter complicate the situation. Strict control measures should be taken by the Veterinary authorities to prevent the slaughtering out of abattoirs. Thus control the spreading of brucellosis infection in human and animals as well as success of eradication programs.

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دراسة مدى تواجد الإصابة بالبروسيلة في بعض المجترات المذبوحة خارج المجازر في محافظة أسيوط

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تم إجراء هذه الدراسة على ٤٨٢ عينة مصل وأنسجة (غدد ليمفاوية وطحال) من ابقار (٢١٥) وجاموس (١٠٣) وأغنام (١٠٥) وماعز (٥٩) مذبوحة خارج المجازر تمثل قري ومدن بشمال وجنوب محافظة أسيوط. تم عمل الفحص السيرولوجي الأولي باختباري الأنتيجين الشريحي المحمض المخمد واختبار الـ روزبنجال فكانت نسب النتائج الأولية للبروسيلة كما يلي: ٢٢ (١٠.٢٣%) في الأبقار و ٣ (٢.٩١%) في الجاموس و ٨ (٧.٦١%) في الأغنام و ٣ (٥.٠٨%) في الماعز. وعند تقييم هذه النتائج باستخدام الإختبارات المؤكدة للبروسيلة مثل إختبار التلزن الانبوبي وإختبار الـ ريفانول وإختبار الـ ايليزا على عدد ٣٦ حيوان . فأعطى إختبار التلزن الانبوبي تأكيدا لـ ٢٢/٢٠ (٩٠.٩%) في الأبقار و ٣/٣ (١٠٠%) في الجاموس وأكد إختبار الـ ريفانول لـ ٢٢/١٩ (٨٦.٣%) في الأبقار و ٣/٣ (١٠٠%) في الماعز في حين أكد إختبار الـ ايليزا على ٢٢/٢١ (٩٥.٤%) في الأبقار و ٣/٣ (١٠٠%) في الجاموس و ٨/٧ (٨٧.٥%) في الأغنام و ٣/٣ (١٠٠%) في الماعز. وأظهر الفحص البكتريولوجي للأنسجة عزل ١١ عترة بروسيلة مليتنسيس نوع حيوي ٣ بحيث كانت ٦ عترات للأبقار وعترة واحدة في الجاموس و ٣ عترات في الأغنام وعترة واحدة في الماعز. تؤكد الدراسة علي ان الحيوانات المذبوحة خارج المجازر تمثل خطرا على متداولي هذه اللحوم حيث انها تحتوى ميكروبات البروسيلة الممرضة كما انها تؤثر سلبا علي برامج مكافحة البروسيلة.