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THE USE OF ELISA FOR DIAGNOSIS AND EPIDEMIOLOGY OF BRUCELLA INFECTION IN SOME FARM ANIMALS IN ASSIUT GOVERNORATE

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SUMMARY

The prevalence of brucella infection among different species of farm animals in Assiut Governorate was estimated by using Rose Bengal plate test (RBPT), Buffered acidified plate antigen test (BAPAT), Tube agglutination test (TAT), Rivanol test (Riv.T.) and Enzyme linked immunosobent assay (ELISA). A total number of 197 cattle blood samples were examined serologically. Brucellosis incidence allover Assiut Governorate was 3.6 %. On the other hand, slaughter houses revealed the highest infection rate (7.1 %) followed by private flocks (1.6 %). While governmental farms did not record any infection rates in this study. A significant correlation between sexually mature animals and the rate of infection was observed. The incidence of brucellosis among cattle was varied according to sex from 0.00 % in males to 6 % in females. Seroprevalence of brucella infection among 129 sheep blood samples was 11.6 % all over the governorate. A highest attack rate (50 %) was observed among the age group 2.3-2.6 years. The incidence of brucella infection was 12.2 % in ewes while no positive cases were recorded in rams. The serological examination of 32 buffaloes' sera did not detect any positive reactors. The role of these animals in transmitting infection to humans was discussed. The public health hazard from detecting brucella antibodies in 7.3 % of the composite milk from 41 dairy cows was also clarified.

INTRODUCTION

Brucellosis is the most important zoonosis in terms of human suffering and is a true zoonosis in that almost all human cases are acquired from animals, in particular goats and sheep. Furthermore, it is considered as the main zoonotic disease especially in the Eastern Mediterranean Region. In Egypt, brucellosis is still remaining one of the major disease problems that affect animal industry as well as animal health inspite of the intensive governmental effort to minimize risk of infection. AOAD (1995) estimated that annual economic losses due to brucellosis were about 60 million Egyptian pounds yearly. Some investigations were carried out in Assiut Governorate to determine the prevalence of brucella infection among some farm animals. Zaghloul and Kamel, (1985) determined an infection rate in 2.7 % of examined cattle. While, Gaddalla (1991) was able to detect brucella infection in 1.7 % of cattle. Abdel-Hafeez et al. (1995) isolated Brucella melitensis biovar 3 from 3 Frisian cows by bacteriological examination of retropharyngeal, suprascupular and supramammary lymph nodes. Moreover, Abdel-Hafeez (1996) determined brucella infection in 0.29 % out of 8774 cattle Sedeek (1999) recorded a brucella seroprevalence of 0.58 % among cattle by using Riv.T. Two years later, Abdel-Hafeez et al. (2001) estimated brucella incidence in 0.53% out of 17456 examined cattle.

On the other hand, Gadalla (1991) estimated an incidence of 0.84 % brucella infection among sheep in Assiut Governorate. While, Ali (1997) recorded an incidence of 1.6 %, 1.6 %, 1.33 %, and 1.4% brucella infection among 21776 examined sheep during the period from January to December 1995 by using BAPAT, RBPT, TAT, and Riv.T respectively. Sedeek (1999) recorded brucella infection in 1.15 %, 1.15 %, 0.85 %, and 1.1 % of 8457 sheep by using BAPAT, RBPT, TAT, and Riv.T respectively. Furthermore, Abdel-Hafeez et al. (2001) estimated an incidence of 1.35 % among 32939 examined sheep.

The incidence of brucellosis among buffaloes was recorded by many authors as El-Olemy (1974); Nashed (1977); Zaghloul and Kamel (1985); Abdel-Wahab (1985) and Abdel-Hafeez et al. (2001) who reported an incidence of 0.27 %, 0.22 %, 1.44 %, 1.8 % and 0.26 % respectively.

The study attempts to investigate the seroprevalence of brucellosis among some farm animals in Assiut Governorate and throw a spot light on the epidemiology of the disease as well as the evaluation of the used serological tests in comparison with ELISA.

MATERIALS and METHODS

The present study was carried out during the period between May, 2003 to June, 2004 in the Department of Animal Hygiene and Zoonoses,

Faculty of Vet. Med., Assiut University; Department of Microbiology and Immunology. Faculty of Med., Assiut University and Assiut Regional Lab. (Animal Health Research Institute).

Source of specimens

A total of 465 animals of different species including cattle (197), sheep (129), and buffaloes (32) were selected for this study. The examined cattle were collected from two governmental farms in Assiut Governorate including Monshaat Khashaba (27), Military farm (25), Private flocks (61), and Slaughterhouses (84). Sheep were randomly collected from private flocks as well as buffaloes samples were collected from slaughterhouses in Assiut Governorate.

2-Collection of samples

A) Blood samples

10 ml of blood were obtained from each examined animals by using a vacutainer tubes. Samples were kept overnight at 4°C to allow the separation of serum then centrifuged at 3000 r.p.m for 10 minutes. The collected sera were coded and kept at - 20°C up to the time of the test.

B) Milk samples:

Milk samples were obtained from 41 dairy cows. Milk serum was obtained according to (Morgan et al., 1978). The clear supernatant was transferred into Eppendorf tube, labeled and preserved at - 20°C up to the time of testing.

Serological examination of collected samples:

All sera were subjected to Rose-Bengal plate test (RBPT) according to Morgan et al. (1969), Buffered acidified plate antigen test (BAPAT) as Alton et al. (1988), tube agglutination test (TAT) according to Alton et al. (1975), Rivanol test (Riv.T) as Alton et al. (1988) and Enzyme linked immunosorbent assay (ELISA) according to Ariza et al. (1992) and Baldi et al. (1996). The used antigens were obtained from the Veterinary Sera and Vaccine Research Institute (VSVRI), Abassia, Cairo, 11517, Egypt. The ELISA kits [Brucella ELISA IgG (Vircell No. G1003)] were obtained from Vircell Company, Web site.

RESULTS

The obtained results were illustrated in Table (1) to Table (9).

Table (1): Seroprevalence of Brucellosis among cattle in Assiut Governorate.

Number of tested blood samples	RBPT				BAPAT				TAT						Riv.T.				ELISA			
	+ve cases		-ve cases		+ve cases		-ve cases		+ve cases		± ve cases		-ve cases		+ve cases		-ve cases		+ve cases		-ve cases	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
197	7	3.6	190	96.4	7	3.6	190	96.4	6	3	1	0.5	190	96.4	7	3.6	190	96.4	7	3.5	190	96.4

Table (2): Ecological distribution of brucella seropositive reactors among cattle in Assiut Governorate.

Locality	No. of tested samples	Positive cases									
		RBPT		BAPAT		TAT		Riv.T.		ELISA	
		No.	%	No.	%	No.	%	No.	%	No.	%
Governmental farms	52	0	0	0	0	0	0	0	0	0	0
Slaughterhouses	84	6	7.1	6	7.1	5	6	6	7.1	6	7.1
Private herds	61	1	1.6	1	1.6	1	1.6	1	1.6	1	1.6
Total	197	7	3.6	7	3.6	6	3	7	3.6	7	3.6

Table (3): Seroprevalence of Brucellosis among cattle according to sex.

Sex	No. of tested samples	Positive cases									
		RBPT		BAPAT		TAT		Riv.T.		ELISA	
		No.	%	No.	%	No.	%	No.	%	No.	%
Female	117	7	6	7	6	6	5.1	7	6	7	6
Male	80	0	0	0	0	0	0	0	0	0	0
Total	197	7	3.6	7	3.6	6	3	7	3.6	7	3.6

Table (4): Age distribution of Brucellosis among cattle.

Age	No. of tested samples	Positive cases									
		RBPT		BAPAT		TAT		Riv.T.		ELISA	
		No.	%	No.	%	No.	%	No.	%	No.	%
1-2 years	51	0	0	0	0	0	0	0	0	0	0
2-3 years	36	0	0	0	0	0	0	0	0	0	0
3-4 years	29	1	3.4	1	3.4	1	3.4	1	3.4	1	3.4
4-5 years	19	2	10.5	2	10.5	1	5.3	2	10.5	2	10.5
5-6 years	28	3	10.7	3	10.7	3	10.7	3	10.7	3	10.7
6-7 years	29	1	3.4	1	3.4	1	3.4	1	3.4	1	3.4
Above 7 years	5	0	0	0	0	0	0	0	0	0	0
Total	197	7	3.6	7	3.6	6	3	7	3.6	7	3.6

Table (5): Results of different serological tests on blood and milk sera of the examined dairy cattle.

Number of tested blood & milk samples	Blood serum										Milk Serum									
	Positive cases										Positive cases									
	RBPT		BAPAT		TAT		Riv.T.		ELISA		WRBPT		WBAPAT		WTAT		WRiv.T.		WELISA	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
41	5	12.2	5	12.2	4	9.8	5	12.2	5	12.2	1	2.4	1	2.4	1	2.4	2	4.9	3	7.3

Table (6): Seroprevalence of Brucellosis among sheep in Assiut Governorate.

Number of tested blood samples	RBPT				BAPAT				TAT						Riv.T.				ELISA			
	+ ve cases		- ve cases		+ve cases		- ve cases		+ ve cases		± ve cases		- ve cases		+ ve cases		- ve cases		+ ve cases		- ve cases	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
129	16	12.4	113	87.6	16	12.4	113	87.6	10	7.8	5	3.9	114	88.4	16	12.4	113	87.6	15	11.6	114	88.4

Table (7): Seroprevalence of Brucellosis among sheep according to sex.

Sex	No. of tested samples	Positive cases									
		RBPT		BAPAT		TAT		Riv.T.		ELISA	
		No.	%	No.	%	No.	%	No.	%	No.	%
Female	123	16	13	16	13	10	8.1	16	13	15	12.2
Male	6	0	0	0	0	0	0	0	0	0	0
Total	129	16	12.4	16	12.4	10	7.8	16	12.4	15	11.6

Table (8): Age distribution of Brucellosis among sheep.

Age	No. of tested samples	Positive cases									
		RBPT		BAPAT		TAT		Riv.T.		ELISA	
		No.	%	No.	%	No.	%	No.	%	No.	%
Up to 1 year	10	0	0	0	0	0	0	0	0	0	0
1.3 - 1.6 years	2	0	0	0	0	0	0	0	0	0	0
1.9 - 2 years	16	6	37.5	6	37.5	3	18.8	6	37.5	6	37.5
2.3 - 2.6 years	10	5	50	5	50	4	40	5	50	5	50
2.9 - 3 years	35	3	8.6	3	8.6	2	5.7	3	8.6	2	5.7
Above 3 years	56	2	3.6	2	3.6	1	1.8	2	3.6	2	3.6
Total	129	16	12.4	16	12.4	10	7.8	16	12.4	15	11.6

Table (9): Summarized table showing brucella positive reactors among some farm animals in Assiut Governorate by using different serological tests.

Species	Total No.	Positive cases									
		RBPT		BAPAT		TAT		Riv.T		ELISA	
		No.	%	No.	%	No.	%	No.	%	No.	%
Cattle	197	7	3.6	7	3.6	6	3	7	3.6	7	3.6
Buffaloes	32	0	0	0	0	0	0	0	0	0	0
Sheep	129	16	12.4	16	12.4	10	7.8	16	12.4	15	11.6

- RBPT: Rose Bengal plate test.
- BAPAT: Buffered acidified plate antigen test.
- TAT: Tube agglutination test.
- Riv.T.: Rivanol test.
- ELISA: Enzyme linked immunosorbent assay.

DISSCUSSION

Brucellosis is still a major disease problem facing the veterinary and medical professions and still imposes serious public health problems in many countries around the world, particularly in the Middle East, Latin America, and the Arabian Gulf as well as economic losses (Abdel-Hafeez et al., 1995 and Saleh et al., 2003).

The prevalence of brucellosis in serologically examined 197 cattle in Assiut Governorate (Table 1) was 3.6 %, 3.6 %, 3 %, 3.6 % and 3.6 % by using RBPT, BAPAT, TAT, Riv.T. and ELISA respectively. These findings were considered higher than those previously recorded by Kaldas, 1990 (0.97 %); Abdel-Hafeez, 1996 (0.3 %) and Abdel-Hafeez et al., 2001 (0.53 %). On the contrary, they are lower than those reported by Hamdy, 1992 (7.5 %); Ibrahim et al., 2002 (29.17 %) ; Ahmed et al, 2002 (26.4 %) and Shalaby et al., 2003 (7.85 %). That difference can be an expected matter due to several factors such as difference in localities, hygienic and control measures adopted, as well as eradication programs in such countries.

The illustrated results in (Table 2) clarify a great variation in the ecological distribution of brucellosis in Assiut Governorate. Maximal infection rate was observed in the slaughterhouses (7.1 %), which may be attributed to the fact that all positive cases for brucella infection are sent to the abattoir as a rule of eradication program (Montasser, 1999 and Montasser et al., 2002). On the other hand, frequent mixing and close contact of animals from different sources in markets, the availability of such herds to be transmitted from infected to a new free area as well as the absence of periodical examination play a significant role in persistence of brucella infection among small private herds amounting to 1.6 % in our study. On the contradictory side, governmental farms did not record any infection rate with brucellosis as they practise to calf hood vaccination with S19 and RB51 vaccines and periodical examination.

The incidence of brucellosis among cattle is variable according to sex from 0.00 % in males to 6 % in females (Table 3). It is difficult to reach any satisfactory conclusion regarding relative sex susceptibility because males and females are kept under such different conditions (Stableforth, 1959).i.e., females in dairy herds appear to be more susceptible than males that are often kept for meat production.

Regarding to the distribution of brucellosis among cattle (Table 4), it is evident that the highest antibody prevalence (10.7 %) was estimated among the age group 5-6 years. This finding supports the view of Shakya et al. (1995); Meyer (1990) and Amin et al. (2004) who reported that brucellosis is the disease of sexually mature animals.

Table (5) elucidates that, 5 (12.2 %) out of 41 examined lactating cows were brucella positive serologically. 3 (7.3 %) of these cases showed brucella agglutinins in their milk by using ELISA test. Shifting to the results of whey agglutination tests, it is clear that the overall results revealed low incidence of brucellosis ranging from 1 (2.4 %) by WRBPT, WBAPAT and WTAT to 2 (4.9 %) by using WRiv.T. This low sensitivity of whey tests may be due to defattening process which may deprive milk whey from some immunoglobulins that present on the surface of fat globules (Sutra et al., 1986). The removal of the solid parts by rennin, change in the pH and the change in molecular weight of the immunoglobulins could be additional factors that led to the low sensitivity of the whey agglutination tests (Sutra et al. 1986) and Hamdy, 1992). WELISA (whey enzyme linked immunosorbent assay) shows a good relative sensitivity and specificity in comparison with the other used agglutination tests and this accord with the results achieved by Nazem et al. (1998) who recommended the use of WELISA as a good substitute to the milk ELISA.

Seroprevalence rates of brucellosis among 129 examined sheep were 12.4 %, 12.4 %, 7.8 %, 12.4 % and 11.6 % by using RBPT, BAPAT, TAT, Riv.T. and ELISA respectively (Table, 6). A lower prevalence was obtained by Bassiony and Ibrahim, (1997) (1.99%); Ali, (1997) (1.4%); Sedeek, (1999) (1.1 %); Abdel-Hafeez et al., 2001 (1.35%) and Shalaby et al. (2003) (8.06%). While, a higher percentage was detected by Shalaby, (1986) (42.75 %) and El-Bauomy, 1989 (15.03 %).

Such variations may be attributed to various factors including the environmental conditions controlling the different areas from which animals were imported. The extent of population movement and the evolutionary changes in the animals' husbandry are considered the main factors which affect the rate of exposure (Shahat, 2000).

The results shown in (Table 7) indicated that the incidence of brucella infection was (12.2 %) in ewes while no positive cases were recorded in rams. These findings nearly coincide with those obtained by Abdel-Ghani et al. (1983) and Masoumi et al. (1992). The high incidence of brucellosis among ewes may be attributed to various factors including the virulence of infecting strain, the size of infecting inoculum, pregnancy, sexual and immune status of the host (FAO / WHO, 1986).

Regarding to the susceptible age to contract the disease among sheep, illustrated results in Table (8) elucidate that a higher infection rate (50 %) was observed among the age group 2.3- 2.6 years. Susceptibility to infection in small ruminants increases after sexual maturity especially with pregnancy. Young animal may be infected but do not show any clinical signs and generally show a weak and transit serological response (Scientific

Committee in Animal Health and Animal Welfare, 2001).

The serological examination of 32 buffaloes' sera did not detect any positive reactors (Table 9). This obtained result upholds that buffaloes are somewhat resistant to the infection (Sadek, 2004). Regarding the results of RBPT and BAPAT (Table 4), it is evident that RBPT and BAPAT detected a higher number of positive reactors among animals compared with TAT. It may be due to the superior sensitivities of these tests which can detect low titers missed by TAT (Davis, 1971; El-Bauomy, 1989; Montasser, 1995 and Shalaby et al., 2003). Moreover, the acidic pH (3.65 in RBPT and 4.0 in BAPAT) of the antigens used in these tests inhibits to a certain extent the activity of non-specific immunoglobulins and enhances agglutination of IgG1 immunoglobulin (FAO / WHO, 1986; Huber, 1989; Alton et al., 1988; Barton, 1994 and Abdel-kader, 1996). The results of Riv.T. are similar to those obtained by RBPT and BAPAT. This confirms that most of the positive reactor animals were in the chronic stage in which IgG1 antibodies are evident. Whereas, Riv.T. is specific for detection of these antibodies after precipitation of IgM and reducing reactivity of IgG2 through the action of Rivanol solution (Mikolon, et al., 1998).

From obtained results in Table (4), it is obvious that Rivanol test is a useful and reliable test in detecting brucellosis without serious number of false positive. It is a highly specific and an official diagnostic test as it detects mainly the presence of IgG through the precipitation of IgM (Hamdy, 1992).

The lower sensitivity of TAT may be attributed to certain limitation of this test especially in the early incubative and in the later chronic stages of the disease (Morgan, 1967; Nicolleti, 1969; Davis, 1971 and Mylrea & Fraser, 1976). Furthermore, this agglutination test failed to detect IgG1 antibodies, since these antibodies can not agglutinate antigen at pH value near neutrality and when in excess these antibodies are liable to block the agglutination activity of other isotypes resulting in false negative reactions (Farina, 1985 and Ahmed et al., 2002).

The used indirect ELISA kits are standardized to provide 98 % sensitivity and 100 % specificity (Vircell Kit, No. G1003). Meanwhile, it requires only a small amount of serum. ELISA reagents are stable and have a long shelf-life. Furthermore, the optical density of ELISA is measured photomtrically and consequently, this eliminates the reader error (Ahmed et al., 2002). From the previously discussed results, it is clear that ELISA provides a rapid, simple, accurate diagnostic assay for brucellosis. This assay will also enable us to assess diagnosis accurately and reduce the eradication time.

Anyhow, the need of a regular investigation must be stressed in order to determine the extent of

brucella infection among farm animals. Control of brucellosis in Egypt should be implanted through organizing a national control programs for combating brucellosis in various domestic animal species allover Egypt or through bilateral projects with some agencies or international organizations aiming to minimize the infection rate among animals and to control the human infection against brucellosis.

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إستخدام إختبار الإليزا لتشخيص الإصابة بعدوى البروسيلا فى بعض الحيوانات الحقلية بمحافظة أسيوط

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إن الإصابة بعدوى البروسيلا بين الفصائل المختلفة للحيوانات الحقلية في محافظة أسيوط قد اختبرت سيرولوجيا باستخدام اختبارات الروزبنجال، الأنتيجين الشريحي المحمض المخمد، التلازن الأنبوبي البطئ، الريفاتول و القياسة المناعية الانزمية (ELISA).و قد تم إجراء الدراسة على 197 من الأبقار و كان معدل الإصابة الكلية في محافظة أسيوط 3.6 %.و سجلت حيوانات المجازر أعلى معدلا للإصابة (7.1%) تلتها القطعان الخاصة (1.6 %) و لم تسجل حيوانات المزارع الحكومية أي معدلات للإصابة في هذة الدراسة. كما لوحظ أيضا وجود علاقة بين الحيوانات البالغة جنسيا و الإصابة. و كان معدل حدوث عدوى البروسيلا بين الأبقار تتراوح من 0.00 % في الذكور إلى 6 % في الإناث. ولقد أشار الفحص السيرولوجي للبروسيلا بين 129 عينة دم من الأغنام إلى معدل إصابة 11.6 % في محافظة أسيوط. و لوحظ أن أعلى معدلا للإصابة كان في المجموعة العمرية (2.3-2.6) سنوات. و على الرغم من أنه لم تسجل أي معدل للإصابة بين الذكور، كان معدل الإصابة بين الإناث 12.2 %.و لوحظ ايضا أن الفحص السيرولوجي لـ 32 عينة جاموس لم يكشف عن أي حالات ايجابية . و قد تم مناقشة الدور الذي تقوم بة هذة الحيوانات لنقل العدوى للإنسان و كذلك اكتشاف الأجسام المضادة لميكروب البروسيلا بنسبة 7.3 % من 41 عينة لبن أبقار حلابة على الصحة العامة.