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EVALUATION OF SOME DIAGNOSTIC TESTS FOR BRUCELLOSIS IN CATTLE.

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SUMMARY

The present study was carried out on 442 cattle of which 238 lactating 176 non lactating 9 aborted cows, 6 calves and 13 bulls of Eriezian breed in a governmental farm at Sharkia Governorate and had a previous history of brucellosis; abortion and retained palcenta.

Blood sera from the examined cattle were subjected to buffered acidified plate antigen (BAPA), Rose Bengal plate (RBPT), Enzyme linked immunosorbent assay (ELISA) Complement fixation test (CFT), Tube agglutination test (TAT). In general, the positive reactors were 28.51 %, 28.05 %, 24.89%, 22.85% and 21.72% while the postitive percentage in lactating cows 29.83 %, 29.41 %, 24.79 %, 22.27 % and 21.85 % while in non lactating cows the percentage of positive reactors were 25.0 %, 22.73 %, 21.02 % and 18.75 % with BAPA, RBPT, ELISA, CFT and TAT respectively.

Application of milk ring test (MRT) on milk from 238 individual lactating cows revealed that 39 (16.39 %) were positivbe reactors.

Relative agreement (concordance) between different serological tests were estimated and discussed.

Trials for isolation of brucella from milk samples using guinea pig inoculation revealed negative results.

INTRODUCTION

Brucellosis is still a severe problem in many countries allover the world, causing major economic losses in livestock and debilitating disease in human beings (Benkirane, 1997).

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Successful control program of brucellosis in animals depends largely on efficient diagnosis of the disease. All serological methods used to detect brucella antibodies are still insufficient to detect all infected cases and no single test is capable of giving conclusive diagnosis in picking up all positive cases (Morgan and Mackinnon, 1979; Salem et al 1987; Wright and Nielsen, 1990 and Tiizard, 1992).

The aim of this work was to carry out:

- A comparative evaluation between some serological assays.

- Determination of the most satisfactory test or tests for routine diagnosis of brucellosis.

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MATERIAL AND METHODS

A herd of Friezian cattle belonged to Sharkia Governorate's farms, with a problem of abortion. This farm had a previous history of infection with brucellosis. 442 serum samples for serological tests, in addition to milk samples from 237 non mastitic lactating cow, not recently parturated for individual milk ring test and guinea pig inoculation for brucella isolation.

Antigens for buffered acidified plate antigen test (BAPA) and milk ring test (MRT) were obtained from United tate, Department of Agriculture, Animal and plant Health Inspection Service, National Veterinary Services Laboratory USA. Antigens for Rose Bengal Plate test (RBPT) and tube agglutination test (TAT) were supplied by the Veterinary Serum and Vaccine Research Institute, Abbassia, Cairo, Egypt. Antigens for complement fixation test (CFT) and lipopolysaccharide antigen (LPS) for Enzyme linked immunosorbent assay (ELISA) were obtained from the Central Veterinary Laboratories, Weybridge, England.

Complement, Haemolysin, Veronal buffer and brucella control serum obtained from Bio-Merieux Laboratory, France. Sheep red blood cells were collected on Alsever solution from healthy brucellosis free male sheep of about 6 months old.

Guinea pigs: brucella free guinea pigs of about 250-350g body weight were used for animal inoculation.

BAPA, RBPT, ELISA, CFT, TAT, MRT, as well as bacteriological examination of milk for brucella isolation, animal inoculation were carried out according to Alton et al (1988). The ELISA assay was read by electronic ELISA reader (Hochest). Results of ELISA were experesed as ELISA unit, samples with 20 units or more were considered positive.

Table (1) Incidence of brucella reactors in cattle using serological tests

Animals	N°	BAPA		RBPT		ELISA		CFT		TAT		MRT	
		N°	%	N°	%	N°	%	N°	%	N°	%	N°	%
Lactating	238	71	29.83	70	29.41	59	24.79	53	22.27	52	21.85	39	16.39
Non-lactating	176	44	25	43	24.43	40	22.73	37	21.02	33	18.75	---	---
Aborted *	9	9	100	9	100	9	100	9	100	9	100	---	---
Bulls	13	2	15.38	2	15.38	2	15.38	2	15.38	2	15.38	---	---
Calves	6	0	0	0	0	0	0	0	0	0	0	---	---
Totals	442	126	28.51	124	28.05	110	24.89	101	22.85	96	21.72	39	16.39

* Samples from aborted cows revealed positive reactors 2- 3 after abortion.

BAPA= Buffered acidified plate antigen test.

CFT = Complement fixation test ; Position reactors at dilution 1 : 4 and higher.

ELISA = Elnzyme linked immunosorbent assay positive reactors at 20 E.U.and higher.

RBPT = Rose bengal plate test .

TAT = Tube agglutination test ; Postive reactors at 1/40 and higher.

MRT = Milk ring test.

Table (2) Serological status of animals tested with milk ring test

No of examined cows	MRT +ve	MRT +ve	MRT -ve	MRT -ve	Total MRT +ve	Total serological +ve
	serological +ve	serological -ve	serological +ve	serological -ve		
238	36	3	34	165	39	70
%	15.13	1.26	14.29	69.33	13.39	29.41

MRT +ve = Positive reactors to milk ring test.

MRT -ve = Negative reactors to milk ring test.

Serological +ve = Positive reactors to one or more serological test.

Serological -ve = Negative reactors to all serological test.

Table (3) Relative agreement between results of different serological tests on examined cattles

Test	Nº of +ve reactors	BAPA	RBPT	ELISA	CFT	TAT
BAPA	126	---	123	109	100	96
	%	---	97.62	86.51	79.37	76.19
RBPT	124		---	107	100	95
	%		---	86.29	80.65	76.61
ELISA	110			---	90	89
	%			---	81.82	80.91
CFT	101				---	79
	%				---	78.22
TAT	96					---
	%					---

Table (4) Correlations of positive reactor cattle to different serological tests

Positive	Reactor cattle		BAPA	RBPT	ELISA	CFT	TAT
	Nº	%	Nº	Nº	Nº	Nº	Nº
5 tests	74	58.27	74	74	74	74	74
4 tests	13	10.24	13	13	13	13	-
4 tests	14	11.02	14	14	14	-	14
4 tests	5	3.94	5	5	-	5	5
3 tests	5	3.94	5	5	5	-	-
3 tests	2	1.57	2	2	-	-	2
3 tests	6	4.72	6	6	-	6	-
3 tests	2	1.57	2	-	2	2	-
3 tests	1	0.79	-	1	1	1	-
3 tests	1	0.79	1	-	1	-	1
2 tests	4	3.15	4	4	-	-	-
Total	127	100	126	124	110	101	96

different serological tests are summarized in Tables (1, 2, 3 and 4).

DISCUSSION

Diagnosis of brucellosis in animals is based mainly on clinico epizootiological situation, serological and bacteriological investigations. Bacteriological diagnosis although it is very important, yet, it is not practicable especially in testing large animal populations, diagnosis has often, depended on serological tests supported where appropriate by bacteriological examination (Alton et al, 1988).

The incidence of brucellosis (Table 1) was 28,51 %, 28,05 %, 24,89 %, 22.85 %, 22.85 %, 21.72 % and 16.39% by BAPA, RBPT, ELISA, CFT, TAT, and MRT respectively. These findings are supported by Jubb et al; (1993) who reported that the incidence of brucellosis in endemic areas may approach 20 - 30 %. In Egypt, the incidence of brucellosis in cattle was 11.2 % in 1939 (Ahmed, 1939), then it fluctuated in the following years and reached to 38.9% in 1989 (Refai, 1989) the incidence of brucellosis became high with the importation of foreign bred cows from different countries where brucellosis was still prevalent, associated with a lack of the proper program of quarantine measures before being introduced into our country on arrival as pointed out by El-Gibaly et al; (1975), Refai (1989).

There was some variation in the incidence of the reactors from one test to another. These differences may be due to variations in procedure and interpretation for each test. Moreover, Wright and Nielsen, (1990) Tizard, (1992) and Quinn et al; (1994) concluded that IgG_1, a very important subisotype could be detected by ELISA, RBPT and MRT but missed by TAT. IgG_2 is a relatively less important subisotype, could be detected by ELISA, TAT, RBPT and MRT, not CFT, IgM which is a cause of false positive, but its importance comes from its presence at very early stages of infection. IgM could be detected by ELISA and TAT but slightly less by RBPT.

The superior sensitivity of BAPA (28.51 %) is a result of the lower final antigen concentration (3.7%) (Alton et al., 1988). Moreover, the final pH of the test (4.0 ± 0.04) after the addition of serum which lies between TAT and the acidity of RBPT can detect both IgG and IgM molecules of specific brucella antibodies (Nelson, 1989) and also permits greater analytical basis in favor of the detection of IgG (Wright and Nielsen 1990). Similar results were reported Amer (1993). Ammar (1995) and Kadry (1996) who reported that BAPA test gave the highest number of positive reactors in diagnosis of brucellosis in cattle and buffaloes. Moreover, (Ali, 1997 and Dawood, 1997) considered BAPA as excellent presumptive test for diagnosis of brucellosis in sheep and goats.

The obtained results revealed a relative high sensitivity of RBPT (28.05 %) this finding agreed with Morgan et al. (1969) who concluded that RBPT is considered more efficient in the detection of both early and chronic brucella infection. In addition to the fact that only 1/10 of the quantity of IgM fraction of immunoglobulin required to give positive results in RBPT (Allan et

al., 1976 and Das and Paranjape, 1988). Also it is suggested that the acidic buffer of RBPT inhibits immunologically the non specific agglutinins (Corbel, 1972, Araj et al., 1988) RBPT detects IgM antibodies formed in the early stage of infection (earlier than other antibodies) more efficiently than IgG_2 antibodies (Beh, 1974; Patterson et, al.; 1976 and Salem et, al., 1987).

Also, the non agglutinating imunoglobulin IgG_1 performs an agglutinating reaction at low pH (Tizard, 1992).

The obtained results agreed with Barsoum et al., (1989), Wright and Nielsen (1990), Amer (1993) Barsoum et, al., (1994) and Mona et, al., (1995) who reported that RBPT test exhibited greater diagnostic sensitivity and specificity than ELISA and TAT during their application on sera of infected animals.

ELISA test revealed 24.89 % positive reactors, the reduction efficacy of ELISA compared with BAPA and RBPT tests may be due to that ELISA is a flexible system as the sensitivity and specificity can be adjusted by changing the physical and chemical conditions of the tests. LPS was reported to adsorb poorly to polystyrene microplate, (Wright and Nielsen, 1987), this agreed with Barsoum et al.; (1995) who reported that competitive ELISA showed moderate to high sensitivity, while Wright and Nielsen (1990) considered the positive and negative reactions by bacteriological examination and challenge was no loss of diagnostic sensitivity or compromise of diagnostic specificity with this assay.

The CFT revealed 22.85 % positive reactors, these findings supported by Ris et al., (1984) and Spencer and Burgess (1984) who concluded that CFT has been reported to be successful in the eradication of brucellosis, however, the test is complicated and has other disadvantages, such as prozone phenomenon, incompatibility with haemolysed or anticomplementary sera and lack of sensitivity. Moreover, Tizard (1992) stated that CFT preferentially detects antibodies of IgG_1 subisotype, but is relatively insensitive to IgM antibodies and fails to detect IgG_2 antibodies, On the otherhand, Saeed and Salem (1980) and Salem et, al., (1987) indicated that CFT is the superior test among the RBPT, and MRT for the diagnosis of bucellosis in cattle.

TAT showed 21.72 % positive reactors, TAT has certain limitation specially in recent and some chronic cases (Nicolas et al.; 1968), some of the suspicious cases in TAT may be truly infected but do not reach the positive titre, also TAT may miss some of infected animals (Davies, 1971 and Fenske, 1977). Table (1) revealed that the TAT is an unsatisfactory test by contemporary standards as it fails to detect some infected animals in early stage or chronic phase when the predominant antibodies belong to isotype with weak agglutinating activity at a neutral pH. The test may be also subjected to false positive reaction caused by non-specific agglutinins which bind to B. abortus cells via the FC region of their immunoglobulin heavy chain structure and by cross reacting antibodies evoked by unrelated bacteria, especially those containing perosamine based epitopes as reported by (Nicolas et, al., 1968; Davies, 1971; Fenske, 1977; Merchant and

Paker, 1983; Brook et al., 1991 and OIE, 1992).

Using MRT in this study revealed that 36 (15.13%) were positive in both MRT and serological tests and 34 (14.29 %) cases were positive in serological tests and negative in MRT, the lower sensitivity of it may be attributed to many factors such as the irregularity in the filtration or excretion of the agglutinins from the blood to the milk (Lembke et, al., 1950) or the appearance of agglutinins in serum at level not enought to be excreted in the quantities sufficient to give positive MRT reaction (Pat and Panigrahi, 1956) or the variation of the fat percentage in the milk of individual animals (Anon, 1958) and the poor tendency of the milk cream to rise up to the top surface to give a positive reaction with MRT even if the amount of agglutinins in the milk present in sufficient amount to induce such reaction (Wilson and Smith 1984) MRT is recommended in herd surveillance test due to its practical and economical, but individual and confirmatory diagnosis serological tests must be used if positive cases were detected. Moreover, Sanders (1989) concluded that the disadvantages of MRT are lack in development of Bang Ring Test., agglutinins are locally produced in the udder of infected animals, this agreed with Kadry (1996). Table (2) showed 3 cases of positive animals in MRT of serologically negative cows. In such cases, early udder infection with brucella organisms could be incriminated and local brucella agglutinins (mainly IgA) are produced and reported to be active in MRT reaction (Thaler et al., 1977 and Tizard, 1992). Similar findings were reported by Roushdy and Moursy (1963), Morgan and Mackinnon (1979) and Kadry (1996).

The relative agreement between results of different tests Table (3) showed higher agreement percentage in reactor samples between BAPA and RBPT (97.62 %). This could be attributed to the nature of the immunoglobulins active in these tests (IgG_1 and IgM). In addition to the variable percentage of relative agreement (78.22 % - 86.51 %), this may be due to the type of immunoglobulins reacted in each test as reported by Allan et al., (1976); Wright and nielsen (1990); Tizard (1992) and Quinn et al., (1994). These results agreed with Amer (1993) and Kadry (1996).

Trials for brucella isolation from samples through its inoculation in guinea pigs revealed negative results. The difficulites of brucella isolation from milk samples of serologically positive animals could be committed to the intermittent shedding of the organisms in milk, limitation of the method used for isolation and localization of virulent strain in other organs (Cameron et al., 1956)., similar findings were reported by Kadry (1996) who reported that no brucella isolates were obtained from milk samples of 16 serologically positive animals.

In conclusion, as shown in Table (4) no test could be reliable alone as reported by Salem et. al., (1978) and Alton et, al., (1988) and the buffered acidified plate Test (BAPA) and the Rose Bengal plate Test (RBPT) remain the most presumptive test for diagnosis of brucellosis together with the

Enzyme Linked Immunosorbent assay (ELISA) and the Complement fixation test (CFT) for the confirmation. The milk ring test (MRT) must be confirmed by some or all of the aforementioned tests.

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