J. Hyg., Camb. (1986), **97**, 457–469
Printed in Great Britain

Evaluation of ELISA in the diagnosis of acute and chronic brucellosis in human beings

By G. F. ARAJ

Department of Microbiology, Faculty of Medicine, Kuwait University, P.O. Box 24923, Kuwait

A. R. LULU, M. Y. MUSTAFA and M. I. KHATEEB

Medical Department, Jahra Hospital, Ministry of Public Health, Kuwait

(*Received 4 March 1986; accepted 10 April 1986*)

SUMMARY

An enzyme-linked immunosorbent assay (ELISA) was used to determine the levels of brucella-specific IgG, IgM and IgA in 173 patients with acute brucellosis, 22 patients with chronic brucellosis and in 281 controls consisting of 98 patients with other infectious etiologies, 20 patients with non-infectious diseases and 163 normal healthy adults. The ELISA results were compared with culture findings, the results of slide agglutination tests with *Brucella melitensis* (M), *B. abortus* (A) and Ross Bengal (RB) antigens, and of tube and microagglutination tests. Brucella cultures were positive in 53 and 5% of patients with acute and chronic brucellosis respectively. The slide agglutination tests with A, M, A plus M and RB antigens were positive in 42, 44, 51 and 98% of patients with acute brucellosis and in 23, 27, 27 and 64% of patients with chronic brucellosis. There was no significant difference in the results between the tube and microagglutination tests regardless of the type of antigen used. At a titre of $\geqslant 80$ or $\geqslant 160$ these tests were positive in 98% and 92% of patients with acute brucellosis and 60 and 40% of patients with chronic brucellosis. The brucella culture and agglutination tests were negative for all the controls.

Brucella ELISA immunoglobulins (Ig) were detected in some individuals in the control groups but the majority of these had titres of $\leqslant 100$ for IgG, IgM, and IgA. However, patients with brucellosis had significantly higher ELISA titres in all classes of Ig than controls but the sensitivity and specificity within each Ig class varied with the titre considered. At a titre of $\geqslant 1600$ the brucella IgG had a sensitivity and specificity of 98% for patients with acute or chronic brucellosis; this decreased with lower reciprocal titres. The brucella IgM titre of $\geqslant 400$ had a sensitivity of 98% and a specificity of 98% for patients with acute brucellosis. However, in patients with chronic brucellosis the brucella IgM was very low. The brucella IgA titre of $\geqslant 200$ showed a sensitivity of 98% and a specificity of 99% for patients with either acute or chronic brucellosis. This study indicates that brucella ELISA is a rapid, sensitive and specific assay, provides a profile of Ig classes in the diagnosis of acute and chronic brucellosis, is useful for mass screening and could be considered the method of choice for the serological diagnosis of brucellosis.

INTRODUCTION

Human brucellosis is still an important infectious disease problem in many parts of the world especially in the Mediterranean countries and the Middle East (FAO/WHO, 1971; Abdussalam & Fein, 1976; Kaufman & Mortone, 1980; Young, 1983; Manes, 1983). The diagnosis depends on a high index of clinical suspicion, culture findings and the demonstration of elevated antibody titres. The varied and non-pathognomonic symptomatic presentation of the disease presents a great challenge to clinicians, especially in the chronic stage in which symptoms are usually vague, the course of illness atypical and the confirmatory diagnostic tests unreliable (Kerr *et al.* 1966; Payne, 1974). Culture of brucella microorganisms from body specimens is time consuming and is often unsuccessful particularly in the chronic form (Wilson & Merrifield, 1951). Thus the diagnosis of chronic brucellosis depends largely on serologic tests (Kerr *et al.* 1968; Alton, Jones & Pietz, 1975; White, 1978; Young, 1983).

Agglutination tests have been the most commonly used. These tests, however, suffer from a high false negative result rate, take 1–2 days to perform, usually require rising titres for diagnosis and cannot per se differentiate the classes of antibodies involved (Kerr *et al.* 1968; Buchanan & Faber, 1980; Klein & Behan, 1981). Tests such as the Coombs anti-human-globulin (Otero, Palenque & Noriega, 1982) complement fixation (Macdonald & Elmslie, 1967) indirect fluorescent antibody (IFA) (Biegeleisen, Bradshaw & Moody, 1962; Edwards, Tannahill & Bradstreet, 1970) and radioimmunoassay (RIA) (Parratt *et al.* 1977) have been used to differentiate immunoglobulin (Ig) classes in patients with brucellosis.

Recently a simple and rapid ELISA test for the determination of brucella-specific IgG, IgM and IgA in serum has shown promising results (Magee, 1980; Gilbert & Hawes, 1981; Sippel, El-Masry & Farid, 1982; De Klerk & Anderson, 1985). We, in addition have reported a high sensitivity and specificity of ELISA when used on cerebrospinal fluid for the diagnosis of patients with neurobrucellosis (Strannegard, Araj & Fattah, 1985; Araj, *et al.* 1986).

This study evaluates the use of ELISA for measuring brucella-specific IgG, IgM and IgA in serum, and defines the limits of titres in relation to culture, slide, tube and microagglutination tests for the diagnosis of patients with acute and chronic brucellosis.

MATERIALS AND METHODS

Study location and population

This study was carried out on patients from Jahra Hospital and Mubarak Al Kabeer Teaching Hospital in Kuwait, during the period from May 1985 through December 1985. A total of 551 serum specimens were obtained, 195 from brucella patients and 356 from control patients, during their first hospital visit and were stored at −20 °C until tested.

Based on objective and subjective assessment of disease history, clinical symptoms, epidemiologic evidence, serologic and culture results the patients and controls were grouped as follows:

Group 1. A total of 173 patients with acute brucellosis. All these patients had

Table 1. *Distribution of patients with brucellosis according to age and sex*

	Number of patients					
	Acute ($N = 173$)		Chronic ($N = 22$)		Total no. (%)	
Age (yrs)	Males ($N = 104$)	Females ($N = 69$)	Males ($N = 16$)	Females ($N = 6$)	Acute	Chronic
0–9	—	—	—	1	—	1 (4)
10–19	22	14	—	—	36 (21)	—
20–29	34	25	1	—	59 (34)	1 (4)
30–39	19	16	3	1	35 (20)	4 (18)
40–49	16	9	6	3	25 (14)	9 (41)
50–59	7	3	2	1	10 (6)	3 (14)
60–69	3	1	2	—	4 (2)	2 (9)
⩾ 70	3	1	2	—	4 (2)	2 (9)
Mean age	36	33	48	36	35	44

a history of drinking raw milk and or contact with goats, cows and camels. They had systemic manifestations compatible with this disease and the diagnosis was confirmed by serology in all and by positive culture results in 91 patients. The distribution of these patients according to age and sex is shown in Table 1.

Group 2. Twenty-two patients suspected of having chronic brucellosis. A history of raw milk ingestion, animal contact or previous infection with brucella over 1 year ago were reported by them. All of these patients had persisting symptoms similar to those described by Kerr *et al.* (1966) and McDevitt (1973) lasting over 1 year. The distribution of these patients according to age and sex is also shown in Table 1.

Group 3. Ninety-eight patients with infectious diseases other than brucellosis. Infection in these patients was due to *Salmonella* sp. (23 patients), *Escherichia coli* (17), *Haemophilus influenzae* (11), *Pseudomonas* sp. (10), *Streptococcus pneumoniae* (8), *Mycobacterium tuberculosis* (7), *Klebsiella* sp. (6), *Listeria* sp. (5), *Toxoplasma* sp. (3), *Neisseria meningitidis* (2), *Staphyloccus aureus* (2), *Treponema pallidum* (2) and *Cryptococcus* sp. (2).

Group 4. Twenty patients with non-infectious diseases. The diseases in these patients were diabetes (9 patients), rheumatoid arthritis (5), systemic lupus erythematosis (4) and leukaemia (2).

Group 5. A total of 162 presumably healthy individuals. These individuals had no history of brucellosis or any unusual febrile illness for the last 2 years. All had been living in Kuwait for a minimum of 4 years. They consisted of hospital (75) and non-hospital personnel (67) of whom 88 were males and 76 females. The mean age was 34 years (range 7–70).

Group 6. Seventy-six sera from British subjects. These specimens were kindly provided by the serology section, St Thomas's Hospital and Medical School, London. They were seronegative for brucella by the slide Rose Bengal and tube agglutination tests. They were used as negative controls to determine the optical density for the end-point titres in the ELISA tests.

Brucella slide agglutination tests

These were carried out using *Brucella melitensis* (M) antigen (Lot No. K 010910) and *B. abortus* (A) antigen (Lot no. K 201710) (Wellcome Diagnostics, England) as well as the Rose Bengal (RB) antigen (Brucelloslide-Test Lot no. 03408, Bio Merieux Laboratories, France) according to the instruction of the manufacturer.

Brucella tube agglutination test (SAT)

The SAT was carried out on doubling dilution of serum from 1 :40 to 1 :1280 using the A and M antigens (Wellcome) according to the instruction of manufacturer.

Brucella microagglutination test (MAT)

The MAT was carried out as described by Bettelheim, Maskill & Pearce (1983) using *B. abortus* strain 1119-3 (courtesy of Dr Brown, National Veterinary Services Laboratories, Ames, Iowa, USA).

Culture of body specimens

The culture and identification of brucella and other microorganisms from body specimens were performed according to established procedures (Lennett *et al.* 1980). The brucella agar medium was supplemented with human plasma negative for brucella antibodies. The brucella cultures were reported as negative after 6 weeks of incubation.

Brucella ELISA

The ELISA antibody titres of brucella-specific IgG, IgM, and IgA in serum specimens were determined as previously described (Araj *et al.* 1986). Briefly, Nunc 96-well microtitration plates were coated with 50 μl of predetermined antigen from a heat-killed *B. melitensis* isolate. After washing, 50 μl serum samples were added to the wells and diluted two-fold from 1/100 to 1/12800. The plates were incubated at 37 °C for 2 h and washed again. Alkaline phosphatase conjugated anti-human IgG or IgM or IgA (Sigma Chemical Co., USA) was added to the wells. The plates were then incubated, washed and P-nitrophenyl phosphate (Sigma) added. The reaction was stopped after 45 min incubation at 37 °C by addition of 3 N sodium hydroxide and the plates were read at 405 nm in a Titertek Multiscan 8-channel spectrophotometer (Flow Laboratories, Scotland). The antibody titre in the test specimen was calculated using cut-off optical densities of two standard deviations above the mean optical density (O.D.) of 76 brucella negative control serum samples from British individuals. Background activity of conjugates and sera was tested for using antigen free-wells, and wells coated with bovine serum albumin (0·010 mg/ml) incubated with conjugate alone, with sera from patients or from controls plus conjugate. Day-to-day variation was determined by testing five positive (at 1/800 dilution) and five negative sera (at 1/100 dilution) daily for 2 months. In each instance the variation in O.D. was less than 10%. Finally, in each run serial dilutions of the same positive (titre 3200) and negative (titre 100) control sera were included in each plate. If the titres of the control sera varied by greater than one dilution step all sera on the plate were tested again.

Table 2. *Recovery of the 92 positive brucella cultures according to time of incubation*

Incubation time and % of positive cultures							
First week		Second week		Third week		Fourth week	
Day	% pos.	Day	% pos.	Day	% pos.	Day	% pos.
1	1	8	26	15	5	22	—
2	—	9	12	16	1	23	—
3	—	10	15	17	2	24	1
4	1	11	4	18	2	25	—
5	3	12	2	19	2	26	1
6	4	13	4	20	4	27	—
7	4	14	2	21	3	28	1
Total	13		65		19		3

Table 3. *Summary of brucella culture and serologic findings in the different groups tested*

	Percentage of positive findings in tested groups				
Test	Acute brucellosis ($N = 173$)	Chronic brucellosis ($N = 22$)	Normals ($N = 162$)	Other infectious diseases ($N = 98$)	Non infectious diseases ($N = 20$)
Culture for brucella	53	5	ND	0	0
Slide agglutination with					
M antigen	44	23	0	0	0
A antigen	42	27	0	0	0
RB antigen	97	64	0	0	0
Tube agglutination (titre $\geqslant 80$)					
A antigen	98	59	0	0	0
Microagglutination (titre $\geqslant 80$)	98	64	0	0	0
ELISA					
IgG titre $\geqslant 1600$	98	100	2	2	0
IgM titre $\geqslant 400$	99	36	4	1	0
IgA titre $\geqslant 200$	98	100	1	0	0

* ND = Not done.

Statistical analysis

Differences in serological test results were assessed by means of the χ^2 test. Significant differences were considered when the probability P was less than 0·05.

RESULTS

Patient population

The male to female ratio was 1:1·5 and 1:3 for the patients with acute and chronic brucellosis respectively. The mean age for the acute cases was 32 yrs (90 % < 40 yrs) and for the chronic cases was 44 yrs (73 % $\geqslant$ 40 yrs) (Table 1).

Table 4. *Distribution of results from patients and controls according to the tube and microagglutination tests*

	No (%) of individuals with indicated titres for					
	Acute brucellosis ($N = 173$)		Chronic brucellosis ($N = 22$)		Controls ($N = 280$)	
Titre	Tube	Micro	Tube	Micro	Tube	Micro
< 40	—	—	7 (31·8)	8 (36·4)	280 (100)	280 (100)
40	4 (2·3)	4 (2·3)	2 (9·0)	—		
80	9 (5·2)	9 (5·2)	3 (13·6)	5 (22·7)		
160	34 (19·7)	17 (9·8)	5 (22·7)	5 (22·7)		
320	33 (19·0)	36 (20·8)	2 (9·0)	3 (13·6)		
640	42 (24·3)	44 (25·4)	3 (13·6)	—		
1280	39 (22·5)	46 (26·6)	—	1 (4·5)		
> 1280	12 (6·9)	17 (9·8)	—	—		

Table 5. *Comparison of results between tube agglutination and microagglutination for the 195 patients with brucellosis*

	No (%) of specimens in		
Difference in titre	Tube higher	Micro higher	Both
None	—	—	73 (37)
One dilution step	41 (21)*	42 (22)	83 (43)
Two dilution steps	9 (5)	23 (12)†	32 (16)
Three dilution steps	2 (1)	5 (3)	7 (4)

* Two specimens showed titre of 40 in micro and 80 in tube.
† Two specimens showed titre of 40 in tube and 160 in micro .

Brucella culture

B. melitensis was recovered from 53 % (91/173) and 5 % (1/22) of patients with acute and chronic brucellosis respectively. The time of culture detection varied from 1–28 days (Mean 11 days) of incubation and the modal recovery was seen between the 8th and 10th day of incubation. Of the 92 positive cultures, 13 % were recovered during the first week, 65 % during the second, 19 % during the third and 3 % during the fourth week (Table 2).

Slide agglutination tests

Using the *B. melitensis* (M) antigen the slide agglutination was positive in 44 and 23 % of patients with acute and chronic brucellosis respectively. Similar results (42 % for acute and 27 % for chronic patients) were obtained using the *B. abortus* (A) antigen. A combination of both tests was positive in 51 % of patients with acute and 27 % with chronic brucellosis. The Rose Bengal (RB) test, however, was positive in 97 and 64 % of patients with acute and chronic brucellosis respectively (Table 3).

All those specimens which were positive by either M or A antigens were also

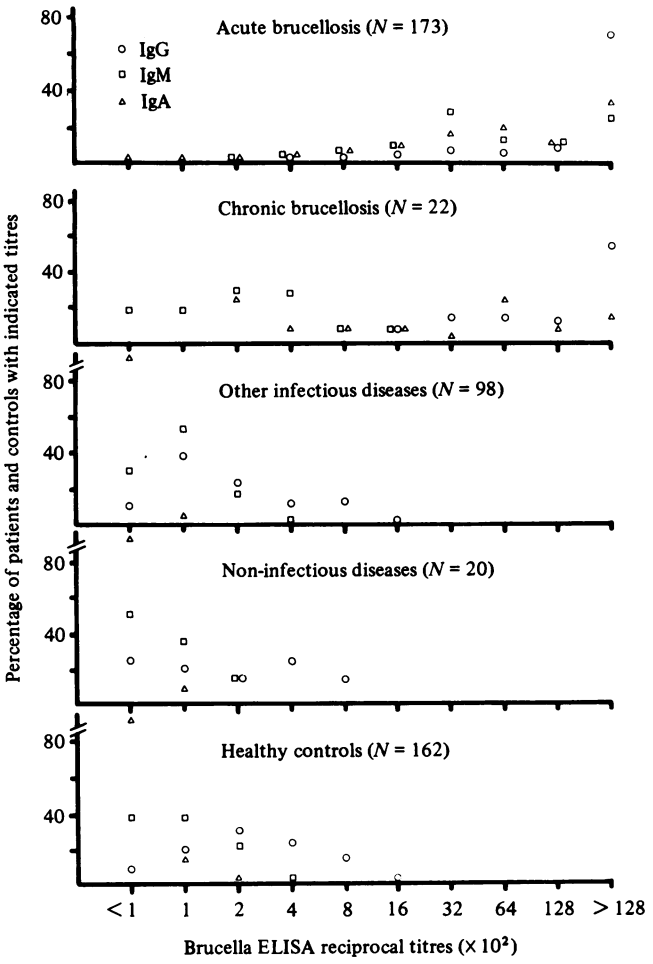

Fig. 1. Distribution of patients with brucellosis and controls according to brucella ELISA titres for IgG, IgM and IgA.

positive by RB antigen. The slide agglutination tests with the three antigens were negative with all the 281 control specimens.

Tube agglutination test (SAT)

A SAT titre of $\geqslant 80$ for the A antigen was obtained in 98% (169/173) and 59% (13/22) of patients with acute and chronic brucellosis respectively (Table 4). There was no significant difference in the results of SAT when patients with acute brucellosis who were culture positive ($N = 91$) were compared with those who were negative ($N = 82$). At the same time 47 of the specimens (23 positive and 24 negative by culture) from patients with acute brucellosis were tested with the M antigen and showed similar positive results (94%) to those tested with A antigen. The SAT negative (titre < 80) specimens in the acute group showed higher titres when retested after 2 weeks. However, there was no change in titre for the SAT negative specimens in the chronic group when similarly retested.

Table 6. *Levels of sensitivity and specificity of various tests findings for patients with brucellosis in relation to controls*

Tests	Reciprocal titre	Predictive values (%) of tests for patients with: Acute brucellosis ($N = 173$) Sensitivity	Acute brucellosis ($N = 173$) Specificity	Chronic brucellosis ($N = 22$) Sensitivity	Chronic brucellosis ($N = 22$) Specificity
Culture	NA	53	100	5	100
Slide agglutination with antigen					
M	*	44	100	23	100
A	*	42	100	27	100
MA	*	51	100	27	100
RB	*	97	100	64	100
Tube agglutination	⩾ 80	98	100	59	100
with A antigen	⩾ 160	92	100	45	100
Micro agglutination	⩾ 80	98	100	64	100
	⩾ 160	92	100	41	100
ELSA					
IgG	⩾ 400	100	64	100	64
	⩾ 800	99	84	100	84
	⩾ 1600	98	98	100	98
	⩾ 3200	94	100	91	100
IgM	⩾ 100	100	36	82	36
	⩾ 200	100	79	64	79
	⩾ 400	99	98	36	98
	⩾ 800	95	100	9	100
	⩾ 1600	87	100	5	100
IgA	⩾ 100	99	89	100	89
	⩾ 200	98	99	100	99
	⩾ 400	98	100	77	100
	⩾ 800	96	100	68	100

NA, not applicable; *, Undiluted.

Microagglutination test (MAT)

The MAT showed similar results to the SAT (Tables 3, 4). Slight differences, however, were observed when comparing the titres obtained in both tests. Eighty per cent of the specimens were within one dilution step difference, 16 % within two and 4 % within three dilution steps (Table 5).

Brucella ELISA

The distribution of brucella-specific IgG, IgM and IgA for the different group of patients and controls are presented in Fig. 1. The reciprocal titres for brucella-specific IgG in the acute and chronic cases were ⩾ 1600 in 97·7 and 100 % of specimens respectively. On the other hand only 2 % of the healthy controls and patients with diseases other than brucella showed such a titre.

Brucella-specific IgM titres of ⩾ 400 were detected in 99 and 35 % of patients with acute and chronic brucellosis respectively and in 2 % of control groups.

Brucella-specific IgA titres of $\geqslant 200$ were detected in 98 and 100% of patients with acute and chronic brucellosis and in $< 1\%$ of control groups.

Sensitivity and specificity of tests

The sensitivity and specificity of various tests in relation to diagnosis of patients with acute ($N = 173$) and chronic ($N = 22$) brucellosis as compared to control groups ($N = 280$) are presented in Table 6.

DISCUSSION

The strict enforcement of the eradication programme among livestock has proven successful for the developed countries in minimizing the incidence of brucellosis (Alausa, 1980; Wise, 1980). The state of Kuwait (population 1·5–1·7 million) is one of the developing countries where the incidence of brucellosis is still on the increase. A total of 732 cases were reported in 1984 with an incidence of 42·78/100000 population as compared to 12 cases in 1976 with an incidence of 1·15/100000 (Health Statistic Annual Report, 1984). In 1985, 1168 cases were reported with an incidence of 68·9/100000 population (personal communication). Investigation of these cases indicated that the infections occurred mainly in rural areas among Bedouins where the traditional drinking of raw milk (goats, camels or cows), the tending of domestic animals or the sharing of accommodation with them provided the main sources of infection – a finding similar to those reported from neighbouring countries (Makarem, Karjoo & Omidi, 1982; Kambal *et al.* 1983). This very substantial increase in reported cases could be partly due to the increased awareness of the disease among clinicians and the availability of improved diagnostic facilities.

Most of the conventional diagnostic tests have proved unreliable for the diagnosis of brucellosis, especially the chronic stage (Kerr *et al.* 1966, 1968; FAO/WHO, 1971; Payne, 1974). In this study, culture yielded positive results in 53 and 5% only of patients with acute and chronic brucellosis. The sensitivity of the slide agglutination tests with M and A antigens was very low for both the acute (40–50%) and the chronic (27%) stages of the disease while the sensitivity of the RB antigen test was 97 and 64%. On the other hand, the sensitivity of SAT was high (98% and 92%) where the titres were > 80 and > 160 in patients with acute disease and was lower (59 and 45%) in patients with chronic brucellosis. These results are consistent with those previously reported (Kerr *et al.* 1966; Payne, 1974; Diaz *et al.* 1978; Kambal *et al.* 1983). The SAT and MAT results were comparable with minor differences only and were similar to the findings of Bettelheim, Maskill & Pearce (1983).

The value of agglutination tests are severely limited by the unacceptably high proportion of false negative results which could be due to a number of factors such as the type of antigen and test used, a prozone phenomenon, class(es) of antibody involved, stage of the disease or to any combination of these (Coghlan & Longmore, 1973; Payne, 1974; Diaz, Maravi-Poma & Rivero, 1976; McNaught *et al.* 1977; Kambal *et al.* 1983). The cut-off SAT titres for positive or negative results may also vary depending on the specimen tested, antigen used and the geographic location of endemic or non-endemic areas (Diaz, Maravi-Poma & Rivero,

1976; Sahs, 1978). Our analyses of SAT results have been based on the most commonly considered positive titres, $\geqslant 80$ and $\geqslant 160$. The specificity was 100% for both titres but the sensitivity decreased in both acute and chronic brucellosis when based upon higher titres. Demonstration of rising titres from two specimens is of greater diagnostic significance than a single titre. In endemic areas, however, a rising titre may not be observed and thus knowledge of the Ig classes, which ELISA provides, would be of great importance.

Attempts to determine Ig classes to brucella in order to identify the stage of the disease (Reddin *et al.* 1965; Wilkinson, 1966; Kerr *et al.* 1966, 1968; Coghlan & Weir, 1967), have been made using methods such as direct agglutination in the presence or absence of 2-mercapto-ethanol, Coombs tests and complement fixation tests. None of these, however, is sufficiently specific (Wilkinson, 1966; Kerr *et al.* 1968; Diaz Maravi-Poma & Rivero, 1976; White, 1978). Direct determination of brucella-specific IgM, IgG and IgA was made possible by the use of primary assays such as IFA (Edwards, Tannahill & Bradstreet, 1970), RIA (Parratt *et al.* 1977) and more recently by ELISA (Magee 1980; Gilbert & Hawes, 1981; De Klerk & Anderson 1985; Strannegard, Araj & Fattah, 1985; Araj *et al.* 1986). These tests avoid the complications of non-agglutinable antibodies. ELISA also avoids the subjectivity in interpreting results of IFA tests and the radioactive hazards of RIA. The antibody conjugates in ELISA have longer shelf lives than IFA or RIA reagents and the test is suitable for screening large number of samples and for automation. Our ELISA results are in agreement with the established findings that in acute (recurrent or current) cases of brucellosis IgM is present in the serum alone or together with IgG and IgA, while in the chronic cases the amount of IgM is minimal or is absent with elevated IgG alone or together with IgA (Coghlan & Longmore 1973; White, 1978; Gilbert & Hawes, 1980; Magee, 1980; Sippel, El-Masry & Farid, 1982). In addition ELISA showed good correlation with the RB and SAT for the diagnosis of acute cases and was superior for the diagnosis of chronic cases of brucellosis.

The limits of normal levels of each Ig class, in ELISA, has to be established for the community tested in each geographic location. This is of special importance for determining the sensitivity and specificity of the ELISA test. Being aware of the endemicity of brucellosis in our community, the sera from British population (where the incidence of brucellosis is negligible) was used to standardize the assay. This was needed in order to establish the actual background antibody levels in our population to avoid false positive results and over diagnosis especially when using ELISA which demonstrated higher sensitivity than conventional tests, a feature noted both in this study and others (De Klerk & Anderson, 1985). The specificity of brucella ELISA varied with the titre. A very high level of specificity for brucellosis was achieved in relation to other infections at titres of $\geqslant 1600$, $\geqslant 400$ and $\geqslant 200$ for IgG, IgM and IgA respectively. The test has also proved to be highly sensitive and specific in the diagnosis of neurobrucellosis using cerebrospinal fluid specimens (Araj *et al.* 1986). One of the controls who had a brucella IgG titre of 1600 suffered from *S. typhi* infection. When tested again after 2 weeks the same brucella titre was obtained indicating no anamnestic reaction. Results from the control groups showed the presence of all Ig classes and there was no significant difference among the three groups for each class of Ig. The normal controls consisted of individuals representing different professions and the presence of

antibody, especially the IgG, in the group reflects the extent of a wide range of exposure to brucella in the community. Among the 4 healthy controls whose sera showed IgG titres of 1600, 2 were from personnel working in a microbiology laboratory and the other 2 were from non-hospital clerks. It is well documented that many persons who are occupationally-exposed to brucellosis have high serological titres but are asymptomatic (McDevitt, 1970, 1973). In a brucella endemic area, such as Kuwait, the difference in Ig levels between the 'high risk individuals' and the rest of the community may not be that distinct. However, the ELISA IgG levels in patients with acute or chronic brucellosis were significantly different from the normal controls. Similar results were also observed in relation to the IgA. The IgM levels, however, showed significant differences between acute and chronic or control groups while it was not significantly different between the chronic and controls. The presence of small amounts of IgM, especially in the control groups, could reflect either overt or subclinical past infection that is no longer active (Coghlan & Weir, 1967; Coghlan & Longmore, 1973). Moreover the fact that IgM levels were low in some patients suspected of having brucellosis, was thought to be due to their having received antibiotics before hospital admission (Magee, 1980; Sippel, El-Masry & Farid, 1982).

Our patients with acute brucellosis had all classes of Ig at the time of their hospital visit. This is in agreement with the findings reported by Sippel, El-Marsy & Farid (1982), from Egypt, but differ from those reported by Reddin *et al.* (1965) and Parratt *et al.* (1977) from England who found that some patients had elevated levels of brucella IgM antibody only. This inconsistency was considered to reflect differences between developed and developing countries in methods used for diagnosis, the habits of the population, and the time taken to make the diagnosis (Sippel, El-Marsy & Farid, 1982). In this study and as noted before (Magee, 1980) none of the sera gave positive results from established methods and negative results from ELISA confirming that ELISA would be an adequate substitute for conventional tests.

In conclusion brucella ELISA is a rapid, sensitive and specific assay with a superior performance over conventional tests. It provides a profile of brucella-specific IgM, IgG and IgA for the diagnosis of patients with acute or chronic brucellosis and is suited for mass screening.

The study was supported by Grant No. BM474178, Health Research Department and Grant No. MI018 Kuwait University. We are indebted to N. V. Madhavan, Jahra Hospital, Nuha Hamdan, Health Research Department, Marie Haj and Sana Mohisien, Department of Microbiology for excellent technical assistance, to T. N. Sugathan, Department of Community Medicine for his statistical advice and to Shashi Humad for her secretarial help.

REFERENCES

ABDUSSALAM, M. & FEIN, D. A. (1976). Brucellosis as a world problem. *Development of Biological Standards* **31**, 9–23.

ALAUSA, O. K. (1980). Brucellosis: socio-economic problems and control in various countries. *Tropical and Geographical Medicine* **32**, 5–11.

ALTON, G. G., JONES, L. M. & PIETZ, D. E. (1975). *Laboratory Techniques in Brucellosis*, 2nd ed. World Health Organization Monograph Series no. 55. Geneva.

ARAJ, G. F., LULU, A. R., SAADAH, M. A., MOUSA, A. M., STRANNEGARD I.-L. & SHAKIR, R. A.

(1986). Rapid diagnosis of central nervous system brucellosis by ELISA. *Journal of Neuroimmunology* (In the Press).

BETTELHEIM, K. A., MASKILL, W. J. & PEARCE, J. (1983). Comparison of standard tube and microagglutination techniques for determining brucella antibodies. *Journal of Hygiene* **90**, 33–39.

BIEGELEISEN, J. JR., BRADSHAW, B. R. & MOODY, M. D. (1962). Demonstration of brucella antibodies in human serum. A comparison of the fluorescent antibody and agglutination techniques. *Journal of Immunology* **88**, 109–112.

BUCHANAN, I. M. & FABER, L. C. (1980). 2-Mercaptoethanol brucella agglutination test: usefulness for predicting recovery from brucellosis. *Journal of Clinical Microbiology* **11**, 691–693.

COGHLAN, J. D. & LONGMORE, H. (1973). The significance of Brucella antibodies in patients in rural area. *Practitioner* **211**, 645–652.

COGHLAN, J. D. & WEIR, D. M. (1967). Antibodies in human brucellosis. *British Medical Journal* **2**, 269–271.

DE KLERK, E. & ANDERSON, R. (1985). Comparative evaluation of the enzyme-linked immunosorbent assay in the laboratory diagnosis of brucellosis. *Journal of Clinical Microbiology* **21**, 381–386.

DIAZ, R., MARAVI-POMA, E., DELGADO, G. & RIVERO, A. (1978). Rose Bengal plate agglutination and counterimmunoelectrophoresis tests on spinal fluid in the diagnosis of Brucella meningitis. *Journal of Clinical Microbiology* **7**, 236–237.

DIAZ, R., MARVI-POMA, E. & RIVERO, A. (1976). Comparison of counter-immunoelectrophoresis with other serological tests in the diagnosis of human brucellosis. *Bulletin of The World Health Organisation* **53**, 417–424.

EDWARDS, J. M. B., TANNAHILL, A. J. & BRADSTREET C. M. P. (1970). Comparison of the indirect fluorescent antibody test with agglutination, complement fixation, and Coombs tests for Brucella antibody. *Journal of Clinical Pathology* **23**, 161–165.

FAO/WHO EXPERT COMMITTEE ON BRUCELLOSIS (1971). World Health Organization technical report series no. 464: 5th report. Geneva.

GILBERT, G. L. & HAWES, L. A. (1981). The antibody response to Brucella: Immunoglobulin measured by enzyme linked immunosorbent assay and conventional tests. *Australian and New Zealand Journal of Medicine* **11**, 40–45.

HEALTH STASTISTICS ANNUAL REPORT (1984). Department of Public Health and Planning, Vital and Health Statistics Division, Ministry of Public Health, Kuwait.

KAMBAL, A. M., MAHGOUB, E. S., JAMJOOM, G. A. & CHOWDHURY N. H. (1983). Brucellosis in Riyadh, Saudi Arabia. A microbiological and clinical study. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **77**, 820–824.

KAUFMAN, A. F. & MORTONE, W. J. (1980). Brucellosis. In *Public Health and Preventive Medicine*, 11th ed. (Ed. M. J. Maxcy-Rosenau), pp. 419–422. New York: Appleton-Century-Croft.

KERR, W. R., COGHLAN, J. D., PAYNE, D. J. H. & ROBERTSON, L. (1966). The laboratory diagnosis of chronic brucellosis. Lancet ii, 1181–1183.

KERR, W. R., MCCAUGHEY W. J., COGHLAN, J. D., PAYNE, D. J. H. QUAIFE, R. A., ROBERTSON, L. & FARRELL, I. D. (1968). Techniques and interpretation in the serological diagnosis of brucellosis in man. *Journal of Medical Microbiology* **1**, 181–193.

KLEIN, G. C. & BEHAN K. A. (1981). Determination of brucella immunoglobulin G agglutinating titre with dithiothreitol. *Journal of Clinical Microbiology* **14**, 24–25.

LENNETT, E. H., BALOWS, A., HAUSLER, W. J. JR. & TRAUNT (1980). *Manual of Clinical Microbiology*, 3rd ed. Washington, D.C., USA. American Society for Microbiology.

MACDONALD, A. & ELMSLIE, W. H. (1967). Serological investigations in suspected brucellosis. Lancet i, 380–382.

MCDEVITT, D. G. (1970). The relevance of the anti-human globulin (Coombs) test and the complement-fixation test in the diagnosis of brucellosis. *Journal of Hygiene*, **68**, 173–187.

MCDEVITT, D. G. (1973). Symptomatology of chronic brucellosis. *British Journal of Industrial Medicine* **30**, 385–389.

MCNAUGHT, D. J., CHAPPEL, R. J., ALLAN, G. S., BOURKE, J. A. & ROGERSON, B. A. (1977). The effect of IgG2 and antigen concentration in the complement fixation test for bovine brucellosis. *Research in Veterinary Science* **22**, 194–197.

MAGEE, J. T. (1980). An enzyme-labelled immunosorbent assay for *Brucella abortus* antibodies. *Journal of Medical Microbiology* **13**, 167–172.

MAKAREM, E. H., KARJOO, R. & OMIDI, A. (1982). Frequency of *Brucella melitensis* in Southern Iran. *Journal of Tropical Pediatrics* **28**, 97–100.

MANES, G. (1983). Epidemiological situation of brucellosis in the Mediterranean countries. *Development of Biological Standards* **56**, 739–747.

OTERO, J. R., PALENQUE, A. F. E. & NORIEGA, A. R. (1982). Microtiter-adapted method that facilitate the Coombs test for brucellosis. *Journal of Clinical Microbiology* **16**, 737–738.

PARRATT, D., NIELSEN, K. H., WHITE, R. G. & PAYNE, D. J. H. (1977). Radioimmunoasssay of IgM, IgG and IgA brucella antibodies. Lancet i, 1075–1078.

PAYNE, D. J. H. (1974). Chronic brucellosis. *British Medical Journal* **2**, 221–222.

REDDIN, J. L. ANDERSON, R. K., JENNESS, R. & SPINK, W. W. (1965). Significance of 7S and macroglobulin Brucella agglutinins in human brucellosis. *New England Journal of Medicine* **272**, 1263–1268.

SAHS, A. L. (1978). Brucellosis (Malta fever; undulant fever). In *Handbook of Clinical Neurology*. Vinken, P. J. & Bruyn, G. W., vol. 33, pp. 305–326. Amsterdam: North-Holland Publishing Company.

SIPPEL, J. E., EL-MASRY N. A. & FARID, Z. (1982). Diagnosis of human brucellosis with ELISA. Lancet ii, 19–21.

STRANNEGARD, I.-L., ARAJ, G. F. & FATTAH, H. A. (1985). Neurobrucellosis in an eight-year-old child. *Annals of Tropical Paediatrics* **5**, 191–194.

WHITE, G. (1978). Immunoglobulin profiles of the chronic antibody response: discussion in relation to brucellosis infection. *Postgraduate Medical Journal* **54**, 595–602.

WILKINSON, P. C. (1966). Immunoglobulin patterns of anti-bodies against Brucella in man and animals. *Journal of Immunology* **96**, 457–463.

WILSON, M. A. & MERRIFIELD, E. V. O. (1951). The antiglobulin (Coombs) test in brucellosis. Lancet ii, 913–914.

WISE, R. I. (1980). Brucellosis in the United States, past present and future. *Journal of American Medical Association* **244**, 2318–2322.

YOUNG, E. J. W. (1983). Human brucellosis. *Reviews of Infectious Diseases* **5**, 821–824.