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Evaluation of Brucellacapt for the diagnosis of human brucellosis

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Acute disease

Summary Objective. To evaluate the role of Brucellacapt in the diagnosis of human brucellosis, and the correlation with the evolution of the disease.

Methods. Twenty-six patients who were admitted to the General Hospital of Albacete (Spain) over a 2-year period and diagnosed with brucellosis were included in the study. One hundred and twenty-three serum samples collected at the time of diagnosis and at intervals during and after treatment were tested by the Coombs test, the standard seroagglutination test (SAT), and Brucellacapt (a new test based on an immunocapture-agglutination technique). To study the specificity of Brucellacapt, sera from 20 patients with other infectious diseases and 20 sera from healthy donors were included in this study.

Results. The sensitivity of the Brucellacapt at the moment of diagnosis was similar to the Coombs test (96 and 100%, respectively), somewhat higher than that of SAT (73%). And the specificity of the Brucellacapt (97.5%) was less than SAT and the Coombs test, that was 100%.

The correlation between the classical serological tests and Brucellacapt, showed that titers in Brucellacapt and Coombs test of patients were both similar in a range of 1-2 dilutions. The correlation between Brucellacapt and Coombs ($r = 0.14$), and between Brucellacapt and SAT ($r = 0.0$) did not reach statistical significance. However, the correlation coefficient between Coombs and SAT was $r = 0.8$.

Conclusions. Brucellacapt and Coombs tests showed a similar sensitivity and specificity in the diagnosis of human brucellosis. In addition, as Coombs test, Brucellacapt could help to diagnose patients with long evolution of brucellosis that are not detected with SAT.

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Introduction

Brucellosis is a zoonosis transmittable to humans worldwide, mainly in the Mediterranean area, the

Middle East, Latin America and Asia.¹⁻⁴ The clinical findings of the disease are non-specific and show great variability.⁵ Thus, the diagnosis requires confirmation via the isolation of the micro-organism, or presence of antibodies against *Brucella* by serological tests.

Blood culture is the most commonly used sample for isolation of *Brucella*. However, the sensitivity of

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culture varies depending on the time evolution of the disease, and the *Brucella* species which causes the infection.⁶⁻⁸ Furthermore, micro-organism culture represents a healthy hazard for laboratory personnel.

A large number of different tests have been used for serological diagnosis of brucellosis, such as Rose Bengal, standard seroagglutination test (SAT) and Coombs tests. The sensitivity of these tests is high, ranging from 65 to 95%.³ However, these methods show an extensive number of limitations. The specificity of these tests in endemic areas for brucellosis decreases because of the high prevalence of anti-*Brucella* antibodies in healthy population.⁹ In patients with a long period of evolution of the disease the immune response could be decreased, giving false negative in the classical serological tests. Similar findings occur in patients with chronic infection, with titers very low, even negative during all the evolution of the disease.¹⁰ On the other hand, patients with relapse are usually diagnosed by the isolation of the micro-organism, or the presence of antibodies titers higher than the last serum sample before the reappearance of symptom. However, these methods have low sensitivity, and they do not allow an early diagnosis of a possible relapse.^{9,10}

In the last year, a new technique to diagnosis human brucellosis has appeared. Brucellacapt is an immunocapture-agglutination technique, which detects all antibodies against *Brucella*. Few studies using this technique have shown very promising sensitivity and specificity.¹¹⁻¹³ However, information concerning the use of this technique in different stages of the disease is scarce.

In this study, we investigate the potential role of Brucellacapt in the diagnosis of human brucellosis compared to the classical serological methods, and the clinical utility of the assay in the evolution of the disease.

Materials and methods

Patients and clinical specimens

Twenty-six patients with brucellosis admitted to General Hospital of Albacete over a 2-year period were included in the study (Table 1). The diagnosis criteria was as follows: (i) isolation of *Brucella* species from blood or other fluid or tissue specimens; (ii) presence of compatible clinical findings (e.g. fever, sweats, arthralgias, hepatomegaly, splenomegaly, or sings of focal disease) together with the demonstration of presence of specific

antibodies to *Brucella* at significant titers, a SAT titer of $\geq 1:160$ or a four-fold rise in SAT or Coombs test titers between two samples collected within 15-30 days of each other, and a Brucellacapt titer $\geq 1:320$.¹² Patients with an evolution time previous to the onset of the disease ≥ 365 days, were considered chronic brucellosis patients.¹⁴

After diagnosis, patients were treated with doxycycline plus streptomycin sulfate or gentamicin, according to internationally accepted treatments.^{14,15} Routine blood culture and serological and clinical evaluations were performed on days 0, 7, 15 and 30 of treatment, at the end of the treatment period, monthly for the first 3 months after the treatment and every third month thereafter for the next 12 months. Identification of *Brucella* spp. was made according to standard microbiological techniques.¹⁶ All isolates were identified as *B. melitensis* biotype 3.

Forty serum samples were used as controls. Among these, 20 samples were from patients with other infectious disease, supported by Dpto. of Microbiology, University of Navarra (*Citrobacter freundii* (2), *Escherichia coli* (7), *Klebsiella oxytoca* (1), *Staphylococcus aureus* (4), *Pseudomonas aeruginosa* (1), *S. mitis* (1), *Salmonella* (2), *Leishmania* (1), *Bacteroides fragilis*(1)) and 20 from healthy donors with no history of brucellosis or exposure to *Brucella*.

Serological methods

SAT, the Coombs anti-*Brucella* test, and Brucellacapt were performed for the 163 serum samples. The SAT and Coombs test were performed with U-shaped microtiter plates instead of tubes. Two-fold serum dilutions were made between 1:20 and 1:40,960 with saline as the diluent (50 μ l per well). These dilutions were then doubled by adding 50 μ l of standardized *Brucella abortus* (Linear Chemicals) diluted in normal saline. SAT reaction were performed after a 24-h incubation at 37 °C. The highest serum dilution showing (> 50% agglutination was considered the agglutination titer. The Coombs test was carried out with the SAT microtiter plates by washing three times with phosphate-buffered saline (pH 7.2) by centrifugation at 3000g for 20 min. This was followed by an energetic inversion of the plates to eliminate the supernatant. After the third washing, 15 μ l of Anti-Human Globulin (Ortho-Clinical Diagnostics) was added to each well. After incubation in a humid chamber for 30 min at 37 °C, the results were read.^{12,17}

Brucellacapt agglutination is similar to that obtained with the Coombs test, determining

Table 1 Titers of Brucellacapt, Coombs and SAT for patients with brucellosis in the basal sample

Patient no.	Diagnosis criteria	Duration of illness at diagnosis (days)	Assays		
			Brucellacapt	Coombs	SAT
1	Sinovial fluid +	390	160	640	80
2	CF ^a	365	320	320	40
3	Blood culture +	20	640	2560	320
4	Blood culture +	3	640	2560	160
5	Blood culture +	45	640	640	320
6	CF	20	640	320	80
7	CF	1095	1280	640	160
8	Arthritis shoulders	20	1280	2560	160
9	Arthritis in shoulders and knees	160	1280	2560	80
10	Sacroiliitis	15	2560	2560	160
11	Arthritis in knees	1095	5120	10 240	< 40
12	Blood culture + (20 days after the onset of the disease)	30	5120	1280	80
13	CF	365	5120	320	40
14	CF	15	5120	20 480	640
15	CF	12	10 240	40 960	40 960
16	CF	20	10 240	2560	1280
17	CF	100	20 480	10 240	640
18	Blood culture +	18	> 40 960	2560	1280
19	CF	390	> 40 960	640	320
20	Blood culture +	3	> 40 960	2560	2560
21	Subclinical brucellosis ^b	5	> 40 960	1280	640
22	CF	50	> 40 960	2560	640
23	Blood culture +	20	> 40 960	1280	320
24	CF	45	> 40 960	10 240	1280
25	Sacroiliitis	20	> 40 960	2560	320
26	CF	15	> 40 960	1280	1280

^a CF, clinical findings (fever, sweats, arthralgias, hepatomegaly, splenomegaly, head-ache, weight lost or sings of focal disease).

^b These patients had a history of ingestion of unpasteurized dairy products and presented malaise and weakness.

antibodies of all classes specific to *Brucella*. The Brucellacapt test (Vircell SL) was performed as specified by the manufacturer. Briefly, 50 µl samples of serum dilutions were added to wells of a U-bottom microtiter plate coated with anti-total human immunoglobulin. Then 50 µl of an antigen suspension (coloured *B. melitensis* bacteria killed by formaldehyde treatment) was added to all the wells. The plates were sealed with adhesive tape and incubated at 37 °C for 24 h in a dark humid chamber. Positive reactions show agglutination over the bottom of the well. Negative reactions are indicated by a pellet at the center of the bottom of the well.¹²

Statistical analysis

Values of the continuous variables are expressed as median and ranges. *P* values, calculated by Epi Info, version 6, were considered to indicate statistical significance when the *P* values were less than 0.05 by two-sided tests.¹⁸

Results

Characteristics of the patients

The diagnosis criteria, duration of illness and serologic findings at baseline, of the 26 patients with brucellosis included in this study are shown in **Table 1** and **Fig. 1**. The median age of patients was 37.5 years (range 17-68), and 20 were male and six female. Patients were treated with a combination of doxycycline and aminoglycosides (streptomycin or gentamicin). The clinical evolution of the patients was favourable and none of them suffered a relapse. Only one patient had positive blood culture 7 days after beginning of treatment. The rest of the samples had negative blood culture.

Sensitivity

The results from the serological tests of 123 serum samples are shown in **Table 2**. These samples were stratified into samples collected at the time of the clinical diagnosis (*n* = 26), during the first (*n* = 13)

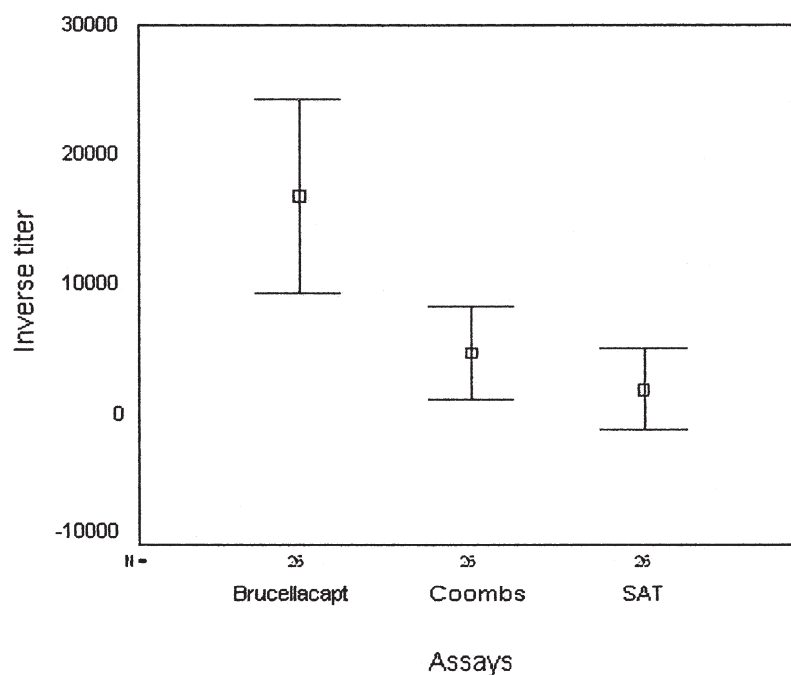


Figure 1 Titers of Brucellacapt, Coombs and SAT for patients with brucellosis in the basal sample.

and the second ($n = 13$) weeks after the beginning of treatment, during the second ($n = 19$) and the third ($n = 15$) months, between the third and sixth months ($n = 18$), and after the sixth month ($n = 19$).

Twenty-five patients were Brucellacapt positive ($\geq 1:320$) on the day of admission (sensitivity, 96%). The Coombs test showed similar sensitivity (100%) that Brucellacapt while SAT showed less sensitivity (73%; $P = 0.049$, two-tailed Fisher's exact test).

Only, one patient was Brucellacapt negative, at the moment of the diagnosis. In this patients, the titer in Brucellacapt was 1:160, negative in SAT (1:80), and positive in Coombs test (1:640), and became Brucellacapt positive in the following serum sample 15 days later (1:40.960). The patient was sinovial fluid culture positive at the onset of the

disease, and had suffered from chronic brucellosis one year before. From the seven SAT negative patients, all of them were Coombs positive and six were Brucellacapt positive. Three out of the six had chronic brucellosis. The period of evolution of the other three patients was 20, 30 and 160 days. However, from the 19 SAT positive patients, only two had chronic brucellosis. The median of evolution time of SAT positive and negative patients was 20 and 365 days, respectively ($P = 0.065$).

The median of serum antibody titers for the 26 patients at admission were as follows: Brucellacapt 1:5120 (range, 1:40 960-1:160), Coombs 1:2560 (range, 1:40 960-1:320), and SAT 1:320 (range 1:2560-1:40), respectively.

Specificity

To study the specificity of Brucellacapt, sera from 20 patients with other infectious diseases and 20 sera from healthy donors was included. No significant differences were found in specificity between Brucellacapt, Coombs and SAT tests. Only one healthy donor had a Brucellacapt titer $> 1:320$ (specificity, 97.5%). All controls were negative in the SAT and Coombs tests (specificity, 100%).

Evolution of serological results in 26 patients with brucellosis

The evolution of antibody titers over time in serum samples from the 26 patients is shown in Fig. 2. The

Table 2 Positivity of brucellacapt, SAT and Coombs test in relation to time after start of treatment in 26 patients

Time evolution (n) ^a	Percentage of positive test ^b (%)		
	Brucellacapt (n) ^a	Coombs (n) ^a	SAT (n) ^a
0 (26)	96 (25)	100 (26)	74 (19)
1-2 weeks (13)	100 (13)	100 (13)	85 (11)
3-4 weeks (13)	100 (13)	92 (12)	46 (6)
2 months (19)	95 (18)	95 (18)	47 (9)
3 months (15)	67 (10)	87 (13)	27 (4)
6 months (18)	61 (11)	72 (13)	22 (4)
>6 months (19)	53 (10)	64 (12)	10 (2)

^a Number of serum samples.

^b We considered positive test: Brucellacapt $\geq 1/320$, Coombs $\geq 1/320$, SAT $\geq 1/160$.

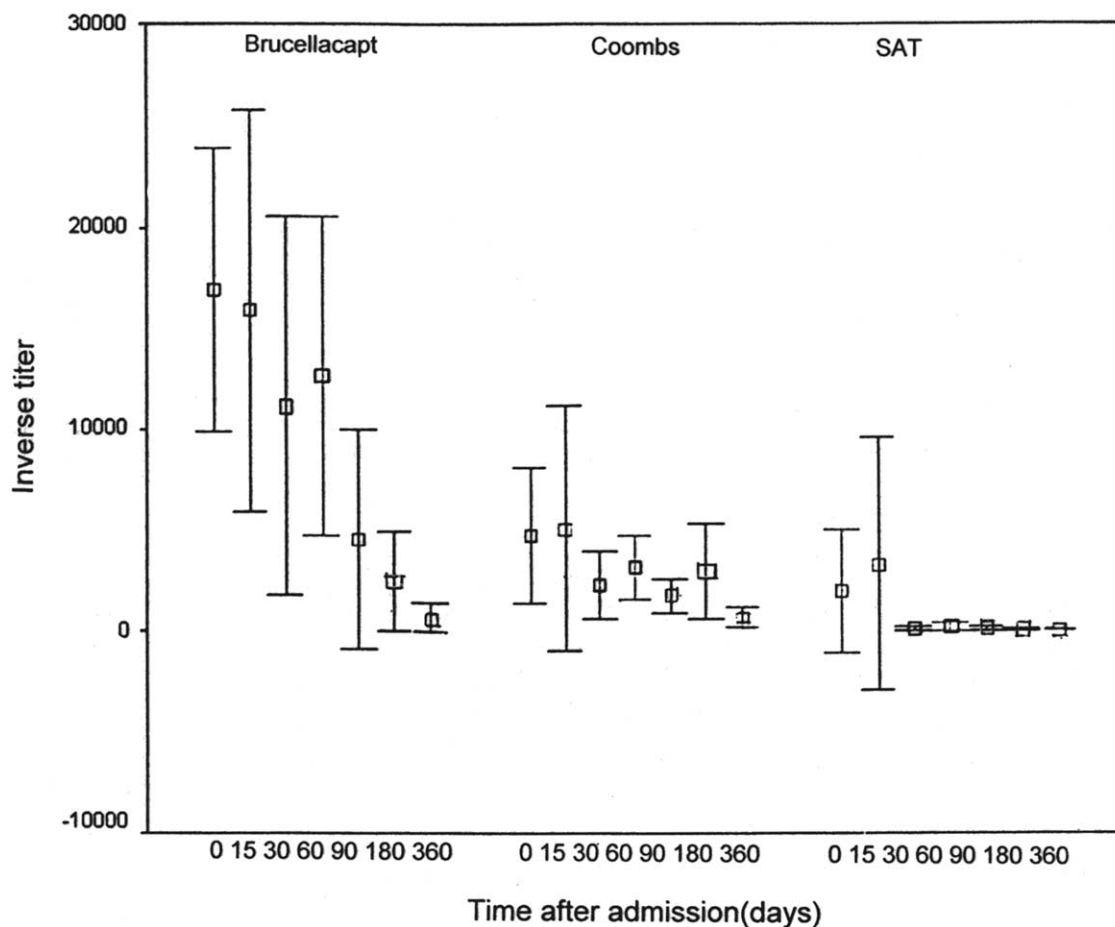


Figure 2 Evolution of serum antibody titer detectable by Brucellacapt, Coombs and SAT, over time in 26 patients with brucellosis.

media of serum titer at the onset of the disease was higher for Brucellacapt than for Coombs and SAT. The first test showing decrease was SAT 30 days after the beginning of treatment with a negative median titer (1:80). Brucellacapt decreased faster than Coombs test, with a median titer of 1:640, 90 days after treatment, and 1:320, 180 days.

However, the percentage of positive patients during the evolution of the disease was similar in Brucellacapt and Coombs test, and lower in SAT (Table 2). Thirty days after diagnosis 100, 92 and 46% of patients were positive in Brucellacapt, Coombs test, and SAT, respectively. Six months after therapy, Brucellacapt and Coombs, were still positive in more than 50% of patients and 10% in SAT.

Correlation between results of classical serological tests and Brucellacapt

The correlation between the classical serological tests and Brucellacapt was studied in the 123 serum samples obtained during the evolution of the

disease. The titers in Brucellacapt and Coombs test of the 123 serum samples are shown in Table 3. Over sixty percent (66.6%) of the patients had similar titers within a range of ± 2 dilutions both tests. The correlation between Brucellacapt and Coombs ($r = 0.14$), and between Brucellacapt and SAT ($r = 0.0$) did not reach statistical significance. However, the correlation coefficient between Coombs and SAT was $r = 0.8$.

Discussion

In this study, we evaluated the usefulness of Brucellacapt in the diagnosis and evolution of human brucellosis. Ninety-six percent of the patients were Brucellacapt positive at the onset of the disease. The only negative patient became positive 15 days after. This sensitivity was similar to Coombs (100%) and higher than SAT (73%).

Groups with long evolution times such as patients with chronic brucellosis and relapse, usually have problems in the diagnosis, due to the low IgM

Table 3 Correlation between Brucellacapt and Coombs titers

Coombs Titer	Brucellacapt											
	<1/40	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10 240	1/20 480	1/40 960
<1/40		1	3	1								
1/40		1	1		2							
1/80			2	2	3							
1/160												1
1/320			1		4	2			2			1
1/640		1		2	3	3	3		5			2
1/1280			1	6	6		3		4			5
1/2560			1	1	1	2	4	3	6	1	1	8
1/5120					1	1		1	4	2	1	
1/10 240					1				2		1	5
1/20 480									1	1		
1/40 960										2		

immune response. In these cases, SAT is usually negative, and the disease is diagnosed by increased IgG and IgA antibodies as detected in the Coombs test.¹⁰ Brucellacapt detects all immunoglobulins against *Brucella*.¹² In this study, the sensitivity and specificity of the test was slightly lower than Coombs, without statistical difference ($P > 0.2$). In addition, both tests detected high titers of anti-*Brucella* antibodies in SAT negative patients with long evolution brucellosis. Similar results have been described by other authors.¹¹⁻¹³ According to these data, the use of Brucellacapt in the diagnosis of human brucellosis could help to detect the disease in patients with long evolution times, which cannot be detected with SAT. Brucellacapt is also easier to perform than Coombs test.

At present, there is no a definite serologic criteria to establish that brucellosis has been cured, since antibody titers in classical serological tests, may remain elevated for a long time after the conclusion of treatment.⁹ A test which could provide information about the evolution of the disease, differentiating cured from sick patients, and relapse from non-relapse patients, would be advantageous. In our group of patients the first test to become negative was SAT, indicating 46% positive patients thirty days after therapy. In contrast, Brucellacapt and Coombs test persisted positive in 100 and 92% of patients, respectively, and 6 months after therapy were still positive in more than 50% of the patients. However, Brucellacapt titers decreased faster than Coombs titers. A prospective study, with relapse and non-relapse patients should be carried out to correlate the Brucellacapt titers with the evolution of the disease, and its utility in the diagnosis of relapses.

There are only a few reports concerning the use of Brucellacapt for diagnosis of human brucellosis.

Gomez et al.¹¹ compared the results obtained by the Coombs test versus Brucellacapt in 112 patients with brucellosis or with brucellar infection suspected on clinical grounds and a history of occupational exposure. They described a direct correlation between both methods ($P < 0.01$). Orduña et al.,¹² studied the utility of the test in the diagnosis of the disease in a group of 82 patients with brucellosis and 412 people living in rural areas with endemic brucellosis. In this paper a $\geq 1:160$ titer threshold was defined as positive and the sensitivity and specificity found was 95.1 and 99%, respectively. In the same work, the sensitivity and specificity for the classical serological tests was: 91.5 and 99.8% for Coombs test; and 65.8 and 100% for SAT, respectively. Subsequently, Serra et al.¹³ described a sensitivity and sensibility of 100 and 95%, respectively, for Brucellacapt in a group of 42 patients with brucellosis.

In conclusion, our results describe a sensitivity and specificity similar for Brucellacapt and Coombs tests, in the diagnosis of human brucellosis, with the advantage of Brucellacapt to be easy to carry out in 24 h without a second step necessary in Coombs test. In addition, as Coombs test, Brucellacapt could help to diagnose patients with long evolution of brucellosis that are not detected with SAT. Both techniques (Brucellacapt and Coombs test) persist positive for long time after therapy in cured patients. What are the practical implications of this test in the serodiagnosis of human brucellosis? Although Brucellacapt can have advantages respect other classical tests, it can hardly replace a rapid screening tests as RB or dipstick¹⁹ in a first diagnostic, because Brucellacapt is more complex, expensive and slow. However, Brucellacapt could be a second level test such as the Coombs test which offers similar sensitivity and specificity.

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