P- ISSN 1991-8941 E-ISSN 2706-6703
2024,(18), (02):16 – 22

Journal of University of Anbar for Pure Science (JUAPS)

Open Access

Extensive Diagnosis of Chronic Brucellosis Using Interferon-γ

Sarah Yahya Abdullah1*, Yasser Mufid Abdul Latif[2], Sawsan Qahtan Taha Al-Quhli[3]

[1, 2, 3] Medical Microbiology Department, Medical College, University of Anbar, Iraq.*
sar21m0007@uoanbar.edu.iq [1]*, dr.yasserjanai@uoanbar.edu.iq[2] , sawsanqt@uoanbar.edu.iq

ARTICLE INFO

Received: 22 / 01 /2024
Accepted: 27/ 03 /2024
Available online: 31/ 12 /2024

10.37652/juaps.2024.146173.1181

Keywords:
Chronic Brucellosis, Interferon -ץ.

ABSTRACT

The clinical presentation of brucellosis may range from subacute to acute or chronic, necessitating extensive tests for a diagnosis. This disease is endemic to Iraq, which brings difficulty in interpreting test data. This study's overarching goal was to develop a diagnostic tool that could aid the immune system's ability to recognize this illness by taking advantage of the variations in interferon-γ. Comparison research was carried out at Anbar Teaching Hospital from October 2022 to September 2023. Eighty-six blood samples from 43 positive Rose Bengal test and 43 control cases were collected in the outpatient infectious clinic. Memory T-lymphocytes create IgM and IgG antibodies, culture, polymerase chain reaction, and interferon-γ that are specific to the *Brucella* antigen. The patients who tested positive came from several communities in the Anbar area and had signs and symptoms of Brucellosis that were later validated using a polymerase chain reaction. For the examined samples, the interferon-γ immunological assay demonstrated a sensitivity of 79.2% and a specificity of 100%. Meanwhile, PCR had a sensitivity of 89.3% and a specificity of 100%. For *Brucell*a-specific IgG, the sensitivity was 21.4% and the specificity was 100%. The sensitivity and specificity of other laboratory studies, such as *Brucella*-specific IgM, were 100%, although *Brucella* culture was positive in just one instance. Compared with *Brucella*-specific IgG, the persistently high levels of interferon-γ in chronic brucellosis may be more helpful for patients undergoing follow-up treatment after acute episodes of low-grade fever.

Introduction:

Interferon gamma (INF-γ) has the potential to be used as an indicator of inflammation due to its role in immunology, which ensures the host's survival and the perpetuation of this ongoing infectious condition. IFN-γ production is caused by brucella parts, such as lipid A, being able to interact with Toll-like receptors to make IL-12 and TNF-α [1]. Brucellosis presents challenges due to its nonspecific symptoms, which can resemble those of other viral illnesses [1,2]. Some of these symptoms include fever, sweating, malaise, and arthralgia, which may not be conclusive for a clinical diagnosis due to their similarity to other illnesses. In addition, serological testing might not differentiate among acute, chronic, or relapsed cases of brucellosis [3,4,5].

*Corresponding author at : Medical Microbiology Department, Medical College, University of Anbar, Iraq
ORCID:https:// https://orcid.org/0000-0000-0000-0000 ,
Tel: +964 7000000000
Email: sar21m0007@uoanbar.edu.iq

The primary stage of *Brucella* infection, known as acute brucellosis, manifests with symptoms such as low-grade fever, perspiration, lethargy, loss of appetite, headache, muscle, and joint pain. Diagnosis may be challenging because these symptoms resemble those of other infectious diseases. Individuals who come in contact with *Brucella* bacteria through handling diseased animals or consuming contaminated meat are at risk of contracting this disease. Inadequate management of acute brucellosis can lead to worsening symptoms and the development of chronic brucellosis, which affects multiple organ systems [5]. Important markers for the diagnosis of acute brucellosis include C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and white blood cell (WBC) count [7; 8; 9]. Elevated CRP level and ESR may indicate an inflammatory response to brucellosis or another infection (among many other possible causes) [9], with patients with acute brucellosis having higher levels than healthy controls. Although

CRP is not a reliable indicator of septicemia on its own, it can be useful in identifying bacterial infections. Acute brucellosis is characterized by an increased WBC count. Infection may trigger neutrophilia [9;10]. To fight off the invading *Brucella* bacteria, the body could make additional white blood cells. Also known as neutrophils acting as the first line of defense against infections, white blood cells could increase in number. Neutrophils are essential when fighting off bacterial infections, such as brucellosis. However, acute brucellosis may cause a drop in lymphocytes, another kind of white blood cell. Lymphocytes may migrate from the healthy tissues and organs in the body to the sick ones, which might explain this decrease. One possible immunological response to brucellosis is an increase in monocytes, the cells that help phagocytose pathogens [11]. During acute brucellosis, the blood cell count may slightly increase. However, standard or specific WBC alone is not sufficient for diagnosing acute brucellosis. Other clinical and laboratory information alongside the WBC count must be evaluated [11]. Many tests can give positive results for brucellosis, including Rose Bengal tests, polymerase chain reaction (PCR) tests, and *Brucella*-specific IgM tests (positive), and *Brucella*-specific IgG tests (negative) [12]. Chronic brucellosis is a prolonged bacterial infection characterized by persistent flu-like symptoms such as fever, lethargy, and joint discomfort. Its detection and treatment may require considerable time, often necessitating prolonged antibiotic therapy [13]. Inflammatory responses to prolonged infections can lead to elevated CRP level and ESR. The seriousness of the sickness may not consistently align with these levels, as they can fluctuate greatly from person to person.

The WBC count can fluctuate widely in individuals with chronic brucellosis, sometimes remaining within normal levels or displaying a slight rise, resembling acute brucellosis. Therefore, relying solely on the WBC count for diagnosing chronic brucellosis is impractical. Positive results are obtained from the Rose Bengal test, PCR, and *Brucella*-specific IgG tests, and the *Brucella*-specific IgM test returns negative results [12].

Stemming from different *Brucella* species, brucellosis can manifest as either a low-grade fever or a milder form known as subacute brucellosis. Less pronounced symptoms such as low body temperature, fatigue, joint pain, or muscle aches may occur, presenting nonspecifically and mirroring other conditions and thereby complicating diagnosis [14]. The CRP level in subacute brucellosis may fluctuate among patients and throughout various phases of the illness. The chronic low-grade inflammation typical of this condition can lead to either slightly raised or within the normal range of CRP level [15]. The WBC count can fluctuate among individuals and throughout various stages of illness. In subacute brucellosis, the WBC count might appear within the normal range or be slightly elevated. Although the ESR generally stays within typical levels, it may demonstrate a notable increase in specific instances. Relapse is characterized by the return of symptoms and acute brucellosis indicators after a brief period of relief and can occur even after effective antibiotic therapy. This phenomenon can be attributed to many variables, one of which is the persistence of the *Brucella* bacteria in certain bodily tissues. Another is the development of tiny, localized abscesses, which may house the bacteria. *Brucella* may live within host cells and avoid detection by the immune system, adding to the risk of recurrence that can happen weeks, months, or even years after the first infection. Relapse symptoms might include systemic indications such as sweating, undulating fever, exhaustion, arthralgia, and other comparable symptoms to the acute illness [16]. CRP levels are commonly increased in patients with relapsing brucellosis due to the immunological response. Although the WBC count varies from patient to patient and illness to infection, it is always increased in recurrent brucellosis [12] and plays an important role in the immunological response. *Brucella*-specific IgM is negative, and the Rose Bengal test, PCR, and *Brucella*-specific IgG are all positive [17].

Material and Method

Cases with a history of acute brucellosis were screened for using a Rose Bengal test, where agglutination was considered a positive result. The following steps were taken to transfer 4 ml of the patient's whole blood to three endotoxin-free heparinized tubes to detect INF-γ:

a. (P): Positive control Mitogen tubes 1 ml

b. (B): Negative or background patient tubes 2 ml

c. (Ag): *Brucella* antigen stimulation tube 1 ml

In brief, 50 µl of the calibrated mixed antigen of *Brucella abortus*, *Brucella melitensis*, and *Brucella suis* were added to the tube (Ag). Tube (P) was already coated with the mitogen phytohemagglutinin for maximum T-cell activation to produce the highest level of INF-γ as a control positive maximum. Nothing was added to the patient's blood in the tube (B) to gain the minimal level of INF-γ that already exists in the patient's blood.

The optimal immune response and avoidance of prozone were achieved by performing a series of dilutional experiments with the *Brucella* antigen. A 20 pg/ml concentration of *Brucella* antigen in 150 ml was the optimal concentration for the study. The same amount of silver was added to the test tube.

Before being promptly placed in an incubator set at 37 °C, the tubes were delicately mixed by flipping them at least five times. Reincubation was carried out for a further 18–20 hours at 37 °C after a remix that was performed 1 hour after incubation. The tubes were spun in a centrifuge at 2000–3000 x g for 10 minutes the next day to isolate the plasma from the red blood cells (RBCs). The obtained plasma was appropriately labeled. Half a milliliter of diluent assay solution was added to each tube's plasma in an ELISA plate well. The liquids were gently mixed after an incubation time of 55 minutes at 37 °C. After incubation, the wells were washed using 300 µl of buffer solution and then reincubated for 55 minutes after adding 50 µl of horseradish peroxidase. After being washed five more times with 300 ml of buffer solution, the cells were added with 100 µl of 3,3′,5,5′-tetramethylbenzidine and allowed to stand in the dark for 25 minutes. The last step was to add 100 µl of a stop solution. The concentration at 450 nm was measured in contrast to 620 nm using an ELISA reader (OD).

.

Table (1) Sequence of size PCR primer and molecular size of PCR products

Gene	Primers Sequence	
16SRNA	F	5'-GAGGCAGCAGTGGGGAATATT-3'
	R	5'-GCCCAGTAAATCCGAACAACG-3'

Table 1 shows the sequence of PCR primers and molecular size of PCR products supplied as a lyophilized product of different concentrations of picomole by the Promega Company and created using data from the National Center for Biotechnology. The sequences used in the investigation for (16rRNA). The infected patients' blood samples were tested for the presence of the *Brucella* genome using these primers.

Results and Discussion

Analysis (Table 2) showed no significant relationship between the IGRA test results and the clinical symptoms of patients with brucellosis including fever in all patients (N= 24), headache (N= 21), and joint pain (N= 9) with P= 0.170. A significant relationship was found between the IGRA test results and the type of brucellosis, which included N= 18 chronic cases, N= 5 subacute cases, and N= 1 acute on chronic with P= 0.361. Significant difference was also observed among the results of IGRA test, Rose Bengal test, ELISA-IgG test, and PCR test. A positive sample (N= 24) was found for the Rose Bengal test, and positive (N= 1) and negative sample samples (N= 23) were found with ELISA-IgG ELISA (P=0.997). PCR test results showed the presence of positive (N= 22) and negative (N= 2) e samples (P= 0.590). This finding indicates that the interferon-gamma is positive in all chronic cases of brucellosis including the subacute, and the acute on chronic brucellosis.

The World Health Organization reports that the actual number of patients with brucellosis is 10–20 times greater than the number of cases recorded. This phenomenon makes it difficult to rely on the Rose Bengal test for diagnosis, even after raising the titer of positivity to 1/160, particularly in an endemic nation such as Iraq. For chronic brucellosis, the Rose Bengal test came back positive in all four severe cases [18] because it does not work with all antibodies for brucellosis and reacts with other tests. Similar to the traditional tube method (Writer's test) not useful in a country such as Iraq where the disease is common [19].

Table (2): Results of the IGRA test percent difference and its relationship to the clinical signs of brucellosis

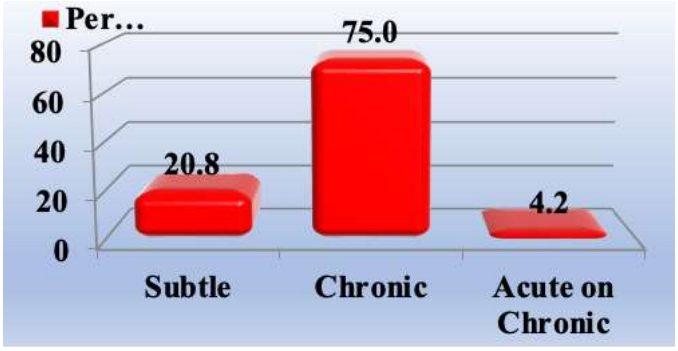
Clinical signs	IGRA test results

		No	Mean±SD
Fever	Yes	24	0.188±0.075
	No	-	-
	P value		-
Headache	Yes	21	0.184±0.074
	No	3	0.210±0.100
	P value		0.594
Joint pain	Yes	9	0.215±0.081
	No	15	0.171±0.069
	P value		0.170
Brucella type	Subacute	5	0.144±0.093
	Chronic	18	0.200±0.070
	Acute on Chronic	1	0.188±
	P value		0.361
Rose Bengal	Positive	24	0.188±0.075
	Negative	-	-
	P value		-
IGRA test final	Positive	19	0.199±0.068
	Negative	5	0.144±0.093
	P value		0.151
ELIZA IgG	<1.0u	1	0.196±
	1.0---	3	0.161±0.054
	2.0---	4	0.175±0.089
	3.0---	4	0.226±0.117
	4.0---	-	-
	5.0---	8	0.179±0.067
	6.0---	2	0.146±0.034
	7.0---	1	0.310±
	8.0---	0	-
	9.0---	0	-
	=>10.0u	1	0.188±
	P value		0.735
ELIZA IgG	Positive (=>9.9995)	1	0.188±
	Negative (<9.9995)	23	0.188±0.077
	P value		0.997
PCR	Positive	22	0.185±0.073
	Negative	2	0.216±0.133
	P value		0.590

#Significant difference between two independent means using Students-t-test at 0.05 level
^Significant difference among more than two independent means using ANOVA-test at 0.05 level

represents the clinical types of 24 patients with brucellosis (Figure 1), including subacute, chronic, and acute on chronic types classified based on the characteristics of the symptoms and the duration and severity of the infection.

The data showed variation in the types of infection for patients with brucellosis. The chronic type developed in more than half of the cases (N=18; 75.0%) whose symptoms lasted more than a year. Some subacute cases were discovered (N=5; 20.8%) who were asymptomatic or with mild symptoms, and one case (4.2%) of the acute on chronic type was recorded. A week after an acute infection, *Brucella*-specific IgM develops and stays positive long after the acute case has healed. It also has a good effect on chronic instances. This finding was later accepted, rendering the test useless for distinguishing between the various clinical manifestations of persistent brucellosis [20]. The *Brucella*-specific IgG test indicated a positive result in the initial infection and the subsequent recurrence. However, distinguishing between the disease's development and reversal would still require two tests. Having a positive *Brucella*-specific IgG result is considered a strategy to start managing brucellosis [21].



Figure(1) Types of infection for patients with brucellosis

Molecular Diagnosis

A molecular diagnosis was performed using a PCR (Figure 2) amplification program to detect 16SrRNA genes for 28 blood samples isolated from the patients suspected of brucellosis and 15 blood samples from the control group.

Brucella abortus was identified in the blood samples using specific primers (16SrRNA) amplified by PCR that produced 200 bp amplicons (Figure 2). PCR came back positive for all four clinical entities, raising the question of whether it is identifying dead or living

bacterial genomes, which is particularly problematic in situations of post-acute and subacute infection. None of the four clinical entities could be distinguished using this test [22].

Given that memory T-cells only last about 120 days (as opposed to memory B-cells' 4- to 10-year lifespans), monitoring their activity is the best way to prove chronic brucellosis. In particular, *Brucella* causes cell-mediated inflammation via INF-γ generation [23]. A test such as the QuantiFERON gamma immunological assay for tuberculosis [24] can be used to make sure that memory T-cells are activated in all cases of chronic brucellosis. The patient may still need treatment because the immune system is actively fighting the infection even after symptoms have subsided [23]. This finding also clarifies the PCR results, which indicated the presence of bacterial genomes in the blood.

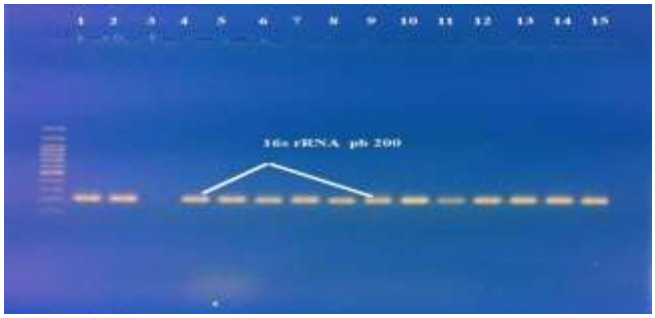


Figure (2) PCR amplification of blood samples from patients suspected of brucellosis using the primer (16S rRNA)

Conclusion

The development of functional memory B-cells is responsible for *Brucella*-specific IgG and shows that the person has been vaccinated against the disease. In cases of relapse, re-infection, or localized chronic infections, these cells may also aid in the detection of acute or chronic inflammatory responses to the bacteria. Testing the memory T-cells' performance may provide an overall picture of the bacterium's presence as persistent inflammation to help follow the pathophysiology of the illness. This finding might alter our perspective on brucellosis care by establishing a treatment regimen that should be continued until the precise INF-γ level reaches zero. The results will also aid in distinguishing between pyrexia with vague symptoms and other generalized causes of this condition.

Conflict of Interest

No potential conflicts of interest exist for any of the authors.

Acknowledgements

We are very appreciative of the cooperation of Lagash Laboratories.

Ethical Approval

The study's validity number, 478, was certified on December 30, 2022, by the University of Anbar's Ethical Committee after thorough due diligence.

References

1. Moriyón, J. M. Blasco, J. J. Letesson, F. De Massis, and E. Moreno. (2023). *Brucellosis* and One Health: inherited and future challenges. *Microorganisms*, vol. 11, no. 8, p. 2070.
2. Laine, C. G., Scott, H. M., & Arenas-Gamboa, A. M. (2022). Human brucellosis: Widespread information deficiency hinders an understanding of global disease frequency. PLoS neglected tropical diseases, 16(5), e0010404.
3. Eko, S. N.; Esemu, A. D. Nota, L. M. Ndip.(2022). A review on *brucellosis* in Cameroon: diagnostic approaches, epidemiology and risk factors for infection," *Advances in Microbiology*, vol. 12, no. 7, pp. 415-442.
4. Sharififar, R., Heidari, K., Mazandarani, M., & Lashkarbolouk, N. (2023). Comparison of the polymerase chain reaction method with serological tests in the diagnosis of human brucellosis. Jundishapur Journal of Microbiology, 16(2).
5. Moreno, J.-M. Blasco, and I. Moriyón. (2022). Facing the human and animal *brucellosis* conundrums: the forgotten lessons," *Microorganisms*, vol. 10, no. 5, p. 942.
6. Mengele, G. M. Shirima, B. M. Bronsvoort, L. E. Hernandez-Castro, and E. A. Cook. (2023). Diagnostic challenges of *brucellosis* in humans and livestock in Tanzania: A thematic review. *CABI One Health*, no., p. ohcs20230001.

7. Turner, T. A. (2011). C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) or both? A systematic evaluation in pediatric ulcerative colitis. *Journal of Crohn's and Colitis*, vol. 5, no. 5, pp. 423-429.
8. Sen, T. Demirdal, and S. A. Nemli. (2019). Predictive value of inflammation markers in *brucellosis*. *Archives of Iranian Medicine*, vol. 22, no. 11, pp. 640-645.
9. Günal, A. K. *(*2014). Comparison between serum neopterin, procalcitonin, high-sensitivity C-reactive protein concentrations and erythrocyte sedimentation rates of patients with brucellosis and extracellular bacterial infections," *Turkish Journal of Biochemistry/Turk Biyokimya Dergisi*, vol. 39, no. 1,.
10. Şirin, B. Aridogani, E. Sesle Çetin, E. (2022). Predictive Value of High-sensitivity C-reactive Protein to Albumin Ratio and Monocyte to High-density Lipoprotein Ratio in Patients with *Brucellosis*. *Jundishapur Journal of Microbiology*, vol. 15, no. 2.
11. Akya, J.-P. Gorvel. (2020). Parameters for Preliminary Diagnosis of *Brucellosis*. *Journal of Blood Medicine*, vol. 11, no. null, pp. 107-113, , doi: 10.2147/JBM.S245513.
12. Xu, E. L. (2020). Improved early detection of focal brucellosis complications with anti-*Brucella* IgG," *Journal of Clinical Microbiology*, vol. 58, no. 10, pp. 10.1128/jcm. 00903-20.
13. Pellegrini, J.-P. Gorvel, and S. Mémet. (2022) .Immunosuppressive Mechanisms in *Brucellosis* in Light of Chronic Bacterial Diseases," *Microorganisms*, vol. 10, no. 7, p. 1260.
14. Sargsyan, *S.* (2014). Acute and chronic brucellosis eleven-year audit from a tertiary hospital in Armenia. *The Journal of Infection in Developing Countries*, vol. 13, no. 05.1, pp. 042S-050S, 2019.
15. Abdollahi, M.; Rasoulinejad, S. J.; Mousavi, F. Fattahi, S. (2009). Serum adenosine deaminase activity and c-reactive protein levels in patients with *brucellosis*. *Iranian Journal of Pathology*, vol. 4, no. 3, pp. 113-117.
16. Adem and A. Duguma. (2020). Characteristics and intracellular life of *Brucella* organism: a review. *J. Microb. Biochem. Technol*, vol. 12, no. 3, p. 431.
17. Zakaria, A. M. (2018). Comparative assessment of sensitivity and specificity of rose bengal test and modified in-house ELISA by using IS711 TaqMan Real Time PCR assay as a gold standard for the diagnosis of bovine *brucellosis*, *Biomedical and Pharmacology Journal*, vol. 11, no. 2, pp. 951-957.
18. Roushan, S. Kazemi, F. F. Rostami, and S. Ebrahimpour. (2014). A study of *brucella* infection in humans. *Crescent J Med Biol Sci*, vol. 1, no. 3, pp. 69-75.
19. Ismail,A. K.; Daood, H. Sami Nouri; Ibtihal Husaien Al Jubory. (2020). Seroprevalence of *Brucellosis* from the city Mosul Iraq. *International Journal of Psychosocial Rehabilitation*, vol. 24, no. 02, pp. 3690-3696, , doi10.37200/IJPR/V24I2/PR200692.
20. Yagupsky, P., Morata, P., & Colmenero, J. D. (2019). Laboratory Diagnosis of Human Brucellosis. Clinical microbiology reviews, 33(1), e00073-19.
21. Gómez, M. C., Nieto, J. A., Rosa, C., Geijo, P., Escribano, M. A., Muñoz, A., & López, C. (2008). Evaluation of seven tests for diagnosis of human brucellosis in an area where the disease is endemic. Clinical and vaccine immunology : CVI, 15(6), 1031–1033.
22. Nimri, N. (2003). Diagnosis of recent and relapsed cases of human *brucellosis* by PCR assay. *BMC Infectious Diseases*, vol. 3, no. 1, p. 5, doi: 10.1186/1471-2334-3-5.
23. Gupta and S. Pellett. (2023). Recent Developments in Vaccine Design: From Live Vaccines to Recombinant Toxin Vaccines," *Toxins*, vol. 15, no. 9, p. 563.
24. Siegel, S. M. Cavanaugh, J. Ku, L. Kawamura, and K. Winthrop. (2018). Specificity of QuantiFERON-TB plus, a new-generation interferon gamma release assay. *Journal of Clinical Microbiology*, vol. 56, no. 12, pp. 10.1128/jcm. 00629-18.

P- ISSN 1991-8941 E-ISSN 2706-6703 *Journal of University of Anbar for Pure Science (JUAPS)* Open Access
2024,(18), (02):16 – 22

مفهوم داء البروسيلات المزمن مقاييس مناعية لانترفيرون كاما

سارة يحيى عبدالله[1] *، ياسر مفيد عبداللطيف[2]، سوسن قحطان طه[3]

قسم الاحياء المجهرية، كلية الطب، جامعة الانبار
1*sar21m0007@uoanbar.edu.iq, 2 dr.yasserjanai@uoanbar.edu.iq , 3 sawsanqt@uoanbar.edu.iq

الخلاصة:

اجريت هذه الدراسة للكشف عن حمى مالطة المزمنة بطريقة جديدة وذلك باستخدام اختبار (IGRA) عن طريق قياس انترفيرون جاما بتحفيز خلايا Tالمناعية وقد جمعت 86 عينة من محافظة الانبار من اكتوبر 2022 الى سبتمبر 2023 مقسمة بين 54 عينة للرجال و 32 عينة للنساء من خلال اجراء فحص روز بنگال حيث كانت 42 حالة منهم ايجابية و 44 حالة سلبية .تم فحص المرضى بواسطة اختبار Rose Bengal الذي تم تأكيده بواسطة التشخيص الجزيئي pcr للجين (16 s RNA) .وتمت مقارنة الحالات بنتائج اختبار (IGRA) للانترفيرون كاما بتحفيز خلايا T باستخدام مستضد البروسيلا .كما تم اجراء الاختبار المناعي للاجسام المضادة (IgG) بواسطة Elizaوكانت النتيجة 9 حالات لديهم اصابة مزمنة و 33 حالة اصابتهم ليست مزمنة. في هذا البحث حيث كانت قيم الحساسية pcr , IGRA, IgG في هذه الدراسة 89% و79.2% و21.4% على التوالي، كانت الاعراض السريرية لـــ42 مصاباً بالروسيلا نسبة ممن يعانون من الحمى 100% و 95.5% لديهم صداع ونسبة 40.5% لديهم آلام المفاصل. بالاضافه الى ذالك اظهر اختبار (IGRA) علاقة معنوية على مستوى p= 0.0001 وايضاً اشارت نتائج الدراسة الى ان الانواع السريرية الثلاث للمصابين بحمى مالطا (الاول تحت الحاد والثاني المزمن والثالث الحاد المزمن) حيث كانت نسبة مرضى النوع الاول 20.8% والنوع الثاني 75% اما النوع الثالث 4.2%.وفي الختام يلقي بحثنا الضوء على التحديات والصعوبات في تشخيص بروسيلا المزمنة مؤكداً اهمية اختبار الانتر فيرون جاما كوسيلة واعدة لتشخيص بروسيلا المزمنة حيث يقيس الاستجابة المناعية لمستضد بروسيلا معين لتحفيز الخلايا t المناعية وقد تم التوصل الى التخفيف المناسب للمستضد وهو (50µl / 20pg)علاوة على ذلك يسهم بحثنا في القاء نظرة فاحصة على تشخيص حمى مالطا المزمنه معللاً بذلك نتائج الـــPCR وIgG Eliza حيث تسهم هذه النتائج في تحسين وتعزيز استراتيجيات التشخيص للمرضى المصابين بالروسيلا ولديهم اعراض سريرية مشابهة لامراض اخرى.