ACTA BIOCLINICA

Artículo Original

Harirzadeh y Col.

Volumen 14, N° 28. Julio/Diciembre 2024
Depósito Legal: PPI201102ME3815
ISSN: 2244-8136

DOI: https://doi.org/10.53766/AcBio/2024.14.28.10

EVALUTION OF *WADDLIA CHONDROPHILA* IN WOMEN WITH RECURRENT MISCARRIAGE USING TAQMAN REAL-TIME PCR

Saeed Harirzadeh [1], Farshid Kafilzadeh[1], Hengameh Zandi[2], Mohammad Kargar[1]

1. Department of Biology, Jahrom Branch, Islamic Azad University, Jahrom, Iran.
2. Department of Microbiology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

CORRESPONDENCE: Dr. Kafilzadeh

EMAIL: f.kafilzadeh@gmail.com, kafilzadehfarshid@gmail.com

ABSTRACT

Background: Abortion is one of the major complications of pregnancy. In only 50% of cases the cause of miscarriage is known. One of the most common causes of abortion is *Waddlia Chondrophila (W. Chondrophila),* but no information is available regarding to its presence in Iran. The purpose of this study was to develop a Taqman real-time PCR assay for the detection of *W. Chondrophila* in biological samples from aborted women. Methods: Clinical samples from women with a history of multiple miscarriages, normal delivery were provided. Standard strain WSU 86–1044 has been provided. The primer and probe *W. Chondrophila* were designed. A positive control was provided based on the PUC57 vector.

Received: 05/05/2024
Accepted: 06/02/2024

ACTA BIOCLINICA

Artículo Original

Harirzadeh y Col.

Volumen 14, N° 28. Julio/Diciembre 2024
Depósito Legal: PPI201102ME3815
ISSN: 2244-8136

DOI: https://doi.org/10.53766/AcBio/2024.14.28.10

Technical performance was checked using a control plasmid. The analytical sensitivity is 5 ng/µl control plasmid DNA. Results: No cross-amplification was observed when testing for other pathogens that could be detected in human vaginitis. Taqman real-time PCR assay showed good reproducibility. This real-time PCR was then applied to 100 vaginal swabs. None of the samples tested positive for Waddlia. Conclusion: This new TaqMan real-time PCR assay represents a diagnostic tool that can be used to further investigate the prevalence of *W. Chondrophila* infection in patient samples.

KEYWORDS: Abortion; pregnancy; Waddlia Chondrophila; Taqman real-time PCR.

EVALUACIÓN DE *WADDLIA CHONDROPHILA* EN MUJERES CON ABORTO ESPONTÁNEO RECURRENTE MEDIANTE PCR TAQMAN EN TIEMPO REAL

RESUMEN

Antecedentes: El aborto es una de las principales complicaciones del embarazo. Sólo en el 50% de los casos se conoce la causa del aborto. Una de las causas más comunes de aborto es *Waddlia Chondrophila (W. Chondrophila),* pero no se dispone de información sobre su presencia en Irán. El propósito de este estudio era desarrollar un ensayo de PCR Taqman en

Received: 05/05/2024
Accepted: 06/02/2024

Volumen 14, N° 28. Julio/Diciembre 2024
Depósito Legal: PPI201102ME3815
ISSN: 2244-8136

DOI: https://doi.org/10.53766/AcBio/2024.14.28.10

tiempo real para la detección de *W. Chondrophila* en muestras biológicas de mujeres abortadas.

Métodos: Se proporcionaron muestras clínicas de mujeres con antecedentes de abortos múltiples y parto normal. Se proporcionó la cepa estándar WSU 86-1044. Se diseñaron el cebador y la sonda W. Chondrophila. Se proporcionó un control positivo basado en el vector PUC57. El rendimiento técnico se comprobó utilizando un plásmido de control. La sensibilidad analítica es de 5 ng/µl de ADN plásmido de control. Resultados: No se observó ninguna amplificación cruzada al analizar otros patógenos que pudieran detectarse en la vaginitis humana. El ensayo de PCR en tiempo real Taqman mostró una buena reproducibilidad. La PCR en tiempo real se aplicó a 100 frotis vaginales. Ninguna de las muestras dio positivo para Waddlia. Conclusiones: Este nuevo ensayo de PCR en tiempo real TaqMan representa una herramienta de diagnóstico que puede utilizarse para investigar más a fondo la prevalencia de la infección por *W. Chondrophila* en muestras de pacientes.

PALABRAS CLAVE: Aborto; embarazo; *Waddlia Chondrophila*; PCR en tiempo real Taqman.

Received: 05/05/2024
Accepted: 06/02/2024

ACTA BIOCLINICA

Artículo Original

Harirzadeh y Col.

Volumen 14, N° 28. Julio/Diciembre 2024
Depósito Legal: PPI201102ME3815
ISSN: 2244-8136

DOI: https://doi.org/10.53766/AcBio/2024.14.28.10

INTRODUCTION

Many factors such as chromosomal abnormalities in the fetus, maternal infection, chronic diseases such as diabetes and thyroid disease, alcohol consumption, tobacco use and drugs are involved in miscarriages [1-5]. The incidence of miscarriage is reported to be between 15-20% worldwide [6, 7]. Unfortunately, only 50% of cases have a known cause [8, 9]. One of these uncertain causes can be infections caused by intracellular bacteria. One of these intracellular bacteria is *Waddlia Chondrophila (W. Chondrophila*), which belongs to Chlamydiales. This bacterium is known to be an important factor in cattle miscarriages. It has also been observed in human miscarriages tissue samples [8, 10].

It is therefore etiologically potential to cause miscarriage and infection in the female reproductive tract. Therefore, it is important to establish a statistical relationship between infection with this bacterium and the recurrent and spontaneous miscarriage. It is also very difficult to detect these intracellular bacteria due to their inability to grow in laboratory environment. For this reason, new molecular methods are needed to detect this bacterium [11].

Recently, with the advancement of the technique, diagnostic pathways have been shifted to real-time PCR, which has greatly assisted in the detection of W. Chondrophila. The 16SrRNA gene-based RT-PCR was developed in 2009 by Goy et al. [12]. This method can replace the time-consuming, difficult and low-efficiency methods commonly used, and the speed and ability to count is an important feature for the study of pathogenesis and drug design.

RecA gene is fully conserved and more distinct than 16SrRNA . The recA gene protein is used as a molecular model for systematic molecular studies of bacteria [13, 14].

Received: 05/05/2024
Accepted: 06/02/2024

ACTA BIOCLINICA

Artículo Original

Harirzadeh y Col.

Volumen 14, N° 28. Julio/Diciembre 2024
Depósito Legal: PPI201102ME3815
ISSN: 2244-8136

DOI: https://doi.org/10.53766/AcBio/2024.14.28.10

Therefore, real-time PCR method can help detect this bacterium. Also, no information is available on the presence of this bacterium in Yazd town and Iran. The purpose of this study was to use Taqman Real time PCR to detection of this bacterium in women with miscarriage and healthy childbirth in order to determine the association between miscarriage and the presence of this bacterium.

MATERIAL & METHODS

1. Sample Collection

In this prospective observational study, 54 clinical specimens were collected from vaginal swabs, cervix, gynecological blood and fetal tissue from hospital. Also 54 healthy women without history of miscarriage was collected. The samples swabs are performed using sterile Dacron swabs. Fetal specimens are stored in PBS buffer medium and in transitional medium for subsequent steps. Clinical samples of women with a history of recurrent miscarriage, normal childbirth was obtained from Shahid Sadoughi Hospital in Yazd city of central of Iran. A standard strain WSU 86–1044 was provided from ATCC.

The sample size was selected based on Daniel's formula:

$$n = \frac{z^2 p(1-p)}{d^2}$$

2. DNA extraction

DNA was isolated from the samples according to the manufacturer's instructions using DNA Isolation Kit I (Roche). First, cells are lysed and proteins are digested by incubation with proteinase K. A special lysis/binding buffer and magnetic glass particles (MGP) are then added. Finally, the cleaned DNA is eluted with 50 µl special buffer and stored at -20°C until laboratory analysis.

Received: 05/05/2024
Accepted: 06/02/2024

ACTA BIOCLINICA

Artículo Original

Harirzadeh y Col.

Volumen 14, N° 28. Julio/Diciembre 2024
Depósito Legal: PPI201102ME3815
ISSN: 2244-8136

DOI: https://doi.org/10.53766/AcBio/2024.14.28.10

3. Determination of IgG and IgM titers of Waddlia chondrophila by ELISA

Unfortunately, there is no commercial kit for this bacterium, so since it is in the Chlamydia family, a commercial ELISA kit was used to detection Anti- IgG and IgM Chlamydia (Euroimmun, Germany).

4. Primer design

Using Primer3 software (Rozen and Skaletsky, 2000), a forward primer WadF (5'- CGGCTACTGTTCTGTATC -3'), and a reverse primer WadR (5- GCGTATAACCCTTTGCTTA - 3') and a Taqman probe (5'- CGGCTACTGTTCTGTATC -3') were selected to amplify a 184 bp fragment of *W. Chondrophila*. The region selected was 100% conserved among Waddliaceae. Primers were also blasted against the nucleotide database of the NCBI website to ensure the absence of significant homology with other microorganism sequences.

5. *W. Chondrophila* plasmid control

In order to construct positive control, the plasmid was designed by Gene Fanavaran. The *W. Chondrophila* sequence (GenBank accession No., CP001928.1) was synthesized and cloned into PUC57 between EcoRI and XbaI restriction enzyme sites (Biomatik, Canada). The constructed PUC57 / *W. Chondrophila* plasmid was confirmed by enzyme digestion and sequencing (data not shown). This vector was transformed into Escherichia coli DH5a strain. Finally, the plasmid was extracted by the YTA plasmid extraction kit (Yekta Tajhiz Azma, Iran) and used as a positive control. The target sequence was amplified with the primers WadF and WadR. The final 25 µl reaction mixture contained 0.4 mM of each primer, 1 U/ml Taq DNA polymerase (Biorad), 5 µl of S5555 PCR buffer containing (20 mM Tris/HCl, 100 mM KCl, 3 mM MgCl2,

Received: 05/05/2024
Accepted: 06/02/2024

ACTA BIOCLINICA

Artículo Original

Harirzadeh y Col.

Volumen 14, N° 28. Julio/Diciembre 2024
Depósito Legal: PPI201102ME3815
ISSN: 2244-8136

DOI: https://doi.org/10.53766/AcBio/2024.14.28.10

400 mM dNTP) and 1 µl *W. chondrophila* DNA.

The PCR was performed according to the following procedure: 5 min at 95°C, 40 cycles at 95°C for 20 s, 55.5°C for 20 s, 72°C for 20 s a gel DNA recovery kit (Zymo Research). Extraction of recombinant plasmid DNA was performed with Plasmid Miniprep Kit (Zymo Research), and the presence of the inserted gene was confirmed by sequencing (Data not shown). Plasmids were then linearized and quantified with a Nano Drop ND-1000 Spectrophotometer. Copy numbers of the cloned gene were derived from the molecular weights of the cloning vector and insert, diluted in 10 mM Tris–HCl, pH 8.0 to generate standards ranging from 101 to 106 molecules and stored at 20 C. Extracted DNA was re-suspended in 50 µL of elution buffer and stored at -20 C. To analyze all vaginal samples (aborted and controls) by Taqman real time PCR, we used 1 µL of each DNA.

6. Real-time PCR assay development

Amplification and detection of PCR products was performed using a real-time PCR cycling system. (Rotor Gene, Germany) during 40 cycles. To determine the optimal concentrations of the primers and probes, various concentrations of the primer and probe sets were evaluated using a qPCR assay. The optimal concentration of primers and probe was assessed with 0.5 µM and 0.025 µM by defining the one that gave the highest recorded fluorescence and the lowest threshold cycle (Ct) that can be defined as the point at which the fluorescence crosses the threshold. The optimal QPCR efficacy was obtained using cycling profile included an initial denaturation at 95 0C for 3 min, then 40 cycles of 10 s at 95 0C and 10 s at 60 0C.

The positive plasmid was used to determine the limits of sensitivity and reproducibility of quantitative PCR. A standard curve was constructed for each

Received: 05/05/2024
Accepted: 06/02/2024

Volumen 14, N° 28. Julio/Diciembre 2024
Depósito Legal: PPI201102ME3815
ISSN: 2244-8136

DOI: https://doi.org/10.53766/AcBio/2024.14.28.10

quantitative PCR by serial dilutions ranging from 101 to 106 plasmid copies/µl. To assess possible false-negative results (related to PCR inhibitors), an inhibition assay was systematically performed on all veterinary samples (3 µl of genomic DNA and 1 µl of each positive control at a concentration of 100 copies). In all experiments, each PCR cycle included a negative extraction control (sterile water) containing 5 µl H2O treated with diethylpyrocarbonate (DEPC) to detect possible contaminating DNA.

7. **Detection of the amplification product**

Ten microliters of each PCR product were electrophoresed on 2.5% agarose gels and then observed under UV illumination.

8. **Determination of Analytical specificity, sensitivity, and reproducibility of the Taqman Real-time PCR**

The specificity of quantitative PCR was tested using DNA isolated from various bacteria commonly associated with vaginitis, such as Chlamydia, Klebsiella pneumonia, Campylobacter Staphylococcus aureus, Mycoplasma and Legionella. Using the positive control plasmid, the analytical sensitivity and the reproducibility of the qPCR was assessed on duplicates with 10- fold dilutions (101–106 copies/reaction). These dilutions were used as quantification standards to construct the standard curve by plotting the plasmid copy number against the corresponding Ct values through which we know the number of copies/ml in the different test samples. To assess the reproducibility, the mean Ct of duplicates obtained in 15 independent runs were compared for each concentration of plasmid DNA. Mean, standard deviation (SD), and coefficient of variation (CV) were calculated in Graphpad.

Received: 05/05/2024
Accepted: 06/02/2024

ACTA BIOCLINICA

Artículo Original

Harirzadeh y Col.

Volumen 14, N° 28. Julio/Diciembre 2024
Depósito Legal: PPI201102ME3815
ISSN: 2244-8136

DOI: https://doi.org/10.53766/AcBio/2024.14.28.10

9. Statistical analysis

Descriptive statistical analysis was used for data presentation. Positive and negative serological data were presented as percent.

The total sample size in this study was 100, of which 54 were controls and 46 were women with history of recurrent or sudden miscarriage. Serum samples of women were used for the ELISA to determine the titers or the presence of anti-Chlamydia antibody.

RESULTS

1. IgG and IgM ELISA

Table1: Seropositivity rate of Anti-Chlamydia in control and case groups

	Control		Target	
	IgM(%)	IgG(%)	IgM(%)	IgG (%)
Positive	0	1(1.8)	1 (2.1)	6 (13)
Negative	54 (100)	53 (98.2)	45 (97.9)	40 (87)

The expected band of *W. Chondrophila* were observed and determined to be 184 bp (figure 1). The specificity of the qPCR assay was 100% when testing DNA isolated from the microorganisms specified in the Method, Section 8, indicating that the specificity of the probes provided a high level of discrimination between the amplicons of the two target bacteria and those of the other guaranteed bacteria tested.

Received: 05/05/2024
Accepted: 06/02/2024

ACTA BIOCLINICA

Artículo Original

Harirzadeh y Col.

Volumen 14, N° 28. Julio/Diciembre 2024
Depósito Legal: PPI201102ME3815
ISSN: 2244-8136

DOI: https://doi.org/10.53766/AcBio/2024.14.28.10

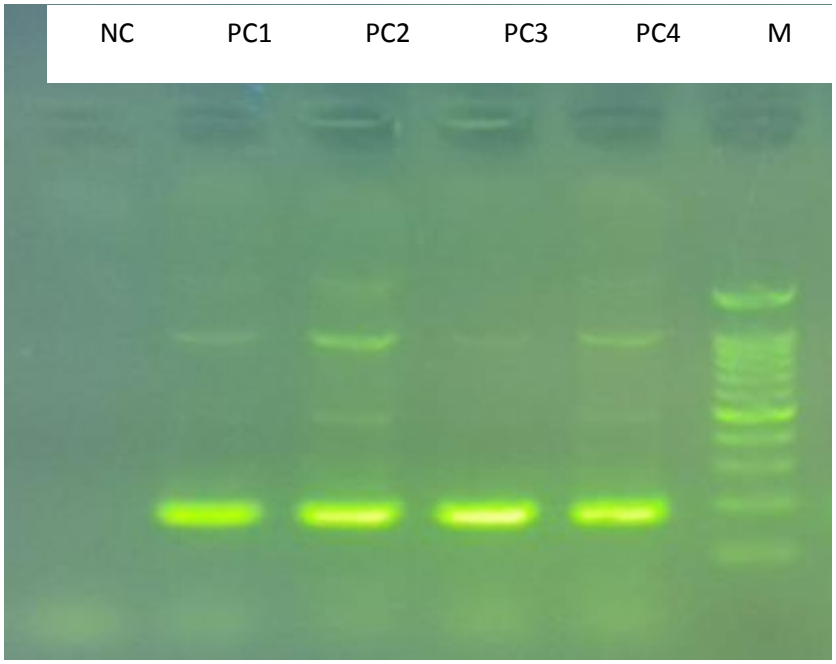


Figure 1. Gel electrophoresis of PCR of W. Chondrophila positive control. NC: Negative Control PC1-4: Positive Samples, M: Molecular Weight

The standard curve of *W. Chondrophila* is shown in Figure 2A and 2B revealing a linear trajectory over 6 logs of plasmid concentration (101 to 107 copies/µL) and curve amplification with a correlation coefficient of 0.998. The average difference between ten-fold dilutions was 3.12 cycles when testing the *W. Chondrophila* plasmid (Figure 2C). The efficiency of the qPCR was found to be 99% for *W. Chondrophila* on Rotor Gene system (Fig. 1 A). Further, it has been shown that the qPCR could detect from 101 to 107 copy of plasmid (Fig. 1 A). Fifteen of 25 replicates (60%) were positive with a *W. Chondrophila* plasmid positive control concentration of 1 copies/µL. We determined that the limit of detection per reaction that could be identified with a 95% probability was 9 10.2 copies/reaction for W. Chondrophila.

Received: 05/05/2024
Accepted: 06/02/2024

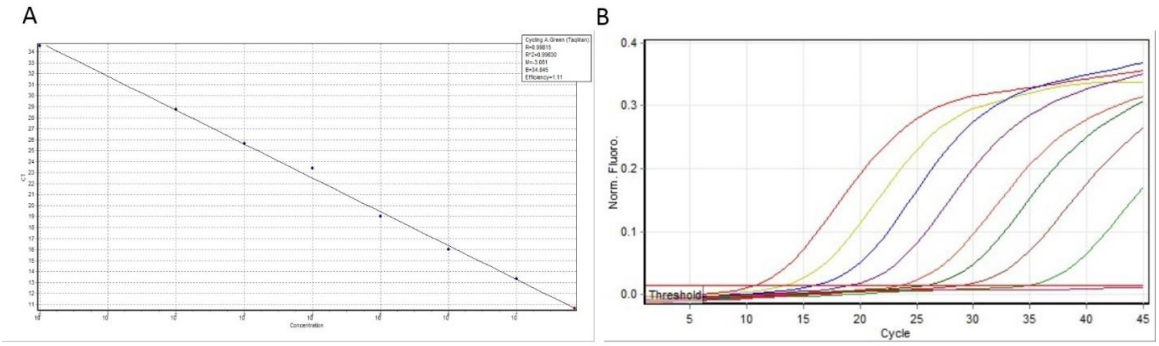
ACTA BIOCLINICA

Artículo Original

Harirzadeh y Col.

Volumen 14, N° 28. Julio/Diciembre 2024
Depósito Legal: PPI201102ME3815
ISSN: 2244-8136

DOI: https://doi.org/10.53766/AcBio/2024.14.28.10



C

No.	Color	Name	Type	Ct	Ct Comment	Given Conc (Copies)	Calc Conc (Copies)
1		Plasmid 1/50	Unknown	10.65			71,000,353
2		1/10	Standard	13.33		10,000,000	9,620,823
3		1/100	Standard	16.00		1,000,000	1,306,278
4		1/1000	Standard	19.01		100,000	137,184
5		1/10000	Standard	23.43		10,000	5,074
6		1/100000	Standard	25.63		1,000	977
7		1/1000000	Standard	28.77		100	94
9		1/100000000	Standard	34.55		1	1
10		NTC	Unknown				

Figure 2. The standard curve of *W. Chondrophila*

DISCUSSION

Recurrent miscarriage is one of the most complex and unpleasant consequences of pregnancy. The exact cause of miscarriage has not yet been fully determined. Intracellular bacterial *W. Chondrophila* is a major cause of miscarriage in cattle [15] as well as other mammals such as humans. It has potential for miscarriage in pregnant women [10]. However, its association with miscarriage has not yet been fully established. Infection detection methods are still evolving and new findings are being made every day to make diagnosis easier and more effective in controlling infection. Methods of amplification of nucleic acids can be performed on high-sensitivity multiple-stranded specimens that are economically viable.

Received: 05/05/2024
Accepted: 06/02/2024

ACTA BIOCLINICA

Artículo Original

Harirzadeh y Col.

Volumen 14, N° 28. Julio/Diciembre 2024
Depósito Legal: PPI201102ME3815
ISSN: 2244-8136

DOI: https://doi.org/10.53766/AcBio/2024.14.28.10

Diagnosis of the bacterium *W. Chondrophila* is important. This bacterium has no known transmission method, it seems to be found in biological specimens such as serum, feces, etc. However, since these microorganisms are difficult to isolate from the culture medium, it is important to find new diagnostic methods because of their low concentration in the medium and resistance to the culture medium. Nowadays, replication methods such as PCR are used to detect *W. Chondrophila*. Finally, with the evolution of quantitative methods, the path was shifted to Q-PCR. The aim of this study was to determine the presence of *W. Chondrophila* in women with miscarriage and healthy delivery in order to determine the relationship between miscarriage and the presence of this bacterium using real time PCR.

In a 2007 study by David Baud et al., investigated the role of *W. Chondrophila* in the prevalence of miscarriage in women with miscarriage and recurrent miscarriage was determined using Western blotting and anti-Waddlia antibody reactivity by immunofluorescence. According to this study, there is a strong relationship between presence of W. chondrophila-specific IgG antibodies and early fetal loss [10]. In 2008, Jorn Siemer and colleagues investigated Chlamydia trachomatis infection as a risk factor for infertility in women in West Africa. Urine samples were analyzed by PCR and ELISA tests were performed on their serum to determine the levels of IgG and IgA antibodies. Overall, 1.6% of infertile women were positive for Chlamydia trachomatis, but similar studies found the diagnostic value and sensitivity of the ELISA test for genital Chlamydia trachomatis infection to be very low; They concluded that serologic tests were not useful to detect chlamydial infections of the genital tract and trachoma [16].

In 2012, Kobe and colleagues examined the detection of *W. Chondrophila* by Immunogenic Proteins. They were looking for reliable, high-throughput

Received: 05/05/2024
Accepted: 06/02/2024

Volumen 14, N° 28. Julio/Diciembre 2024
Depósito Legal: PPI201102ME3815
ISSN: 2244-8136

DOI: https://doi.org/10.53766/AcBio/2024.14.28.10

serological methods. To do this, they used a combination of genomic and proteomic methods. They used two new proteins, Wim3 and Wim4 (recombinant proteins expressed in Escherichia coli) as antigens in an ELISA method. They concluded that these immunogenic proteins could be used in serological tests [17].

In 2014, David Baud, and his colleagues investigated the role of *W. Chondrophila* infection in the placenta and its association with miscarriage using PCR and immunohistochemically methods. Their results showed that there was a strong association between *W. Chondrophila* infection and miscarriage in women. They suggested that when you suspect miscarriage associated with W. Chondrophila, it is recommended that PCR be performed on placenta and vaginal swab specimens [18].

In 2017, Baud et al. in Switzerland investigated the effects of sperm infection on infection with *W. Chondrophila* using methods such as immunohistochemistry and real time PCR. In this study, sperms were artificially infected. They reported that this bacterium had significant negative effects on the function and structure of human sperm DNA [19].

CONCLUSION

In this study, *W. Chondrophila* was optimized in terms of sensitivity and specificity. This method allowed the detection of Waddlia DNA. Given the evidence that this bacterium is an important contributor to miscarriage, Taqman real time PCR was inserted for 46 samples of vaginal and cervical swabs and blood of women and fetal tissue. But *W. Chondrophila* DNA was not detected in either of samples with recurrent miscarriage. Therefore, it can be concluded that Taqman real time PCR is a highly sensitive and specific method for the detection of *W. Chondrophila* in clinical development. This study was the first study in relationship with W. Chondrophila, therefore we had more limitation including the no presence of

Received: 05/05/2024
Accepted: 06/02/2024

Volumen 14, N° 28. Julio/Diciembre 2024
Depósito Legal: PPI201102ME3815
ISSN: 2244-8136

DOI: https://doi.org/10.53766/AcBio/2024.14.28.10

standard strain for the clinical evaluation, the no presence the specific test such as serology and standard molecular kits. Therefore it is better that it used to more specimens because the low frequency of this bacterium. Also Taqman real-time PCR method in this study was high sensitivity but it is need for more specimens and use of another genes of this bacterium in same methods.

Author's contributions

In this study, all authors contributed to the design, write, and review of the manuscript. Saeed Harirzadeh contributed to data collection and biological tests. Farshid Kafilzadeh managed and supervised the experiments and results.. Dr. Hengameh Zandi carried out statistical analysis of data. Mohammad Kargar did PCR and analysis.

Conflict of Interests

There is no Conflict of Interests

Funding/Support

There is no funding statement.

REFERENCES

[1] Carp H. Progestogens and pregnancy loss. Climacteric. 2018; 21: 380-384.

[2] Goodarzi P, Falahzadeh K, Aghayan H, Payab M, Larijani B, Alavi-Moghadam S, et al. Therapeutic abortion and ectopic pregnancy: alternative sources for fetal stem cell research and therapy in Iran as an Islamic country. Cell and Tissue Banking. 2019; 20: 11-24.

[3] Kleinman ME, Brennan EE, Goldberger ZD, Swor RA, Terry M, Bobrow BJ, et al. Part 5: adult basic life support and cardiopulmonary resuscitation quality: 2015 American Heart Association guidelines update for cardiopulmonary resuscitation and

Received: 05/05/2024
Accepted: 06/02/2024

Volumen 14, N° 28. Julio/Diciembre 2024
Depósito Legal: PPI201102ME3815
ISSN: 2244-8136

DOI: https://doi.org/10.53766/AcBio/2024.14.28.10

emergency cardiovascular care. Circulation. 2015; 132: S414-S435.

[4] Pillai RN, Konje JC, Richardson M, Tincello DG, Potdar N. Prediction of miscarriage in women with viable intrauterine pregnancy—A systematic review and diagnostic accuracy meta-analysis. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2018; 220: 122-131.

[5] Wahabi HA, Fayed AA, Esmaeil SA, Bahkali KH. Progestogen for treating threatened miscarriage. Cochrane Database of Systematic Reviews. 2018; : .

[6] Kaandorp SP, Lauw MN, van der Schoot CE, Goddijn M, van der Veen F, Koene HR, et al. Prevalence of JAK2V617F mutation in women with unexplained recurrent miscarriage. Journal of Thrombosis and Haemostasis. 2010; 8: 2837.

[7] Regan L, Rai R. Epidemiology and the medical causes of miscarriage. Best practice & research Clinical obstetrics & gynaecology. 2000; 14: 839-854.

[8] Baud D, Greub G. Intracellular bacteria and adverse pregnancy outcomes. Clinical Microbiology and Infection. 2011; 17: 1312-1322.

[9] Carrington B, Sacks G, Regan L. Recurrent miscarriage: pathophysiology and outcome. Current Opinion in Obstetrics and Gynecology. 2005; 17: 591-597.

[10] Baud D, Thomas V, Arafa A, Regan L, Greub G. Waddlia chondrophila, a potential agent of human fetal death. Emerging infectious diseases. 2007; 13: 1239.

[11] Lamoth F, Pillonel T, Greub G. Waddlia: an emerging pathogen and a model organism to study the

Received: 05/05/2024
Accepted: 06/02/2024

Volumen 14, N° 28. Julio/Diciembre 2024
Depósito Legal: PPI201102ME3815
ISSN: 2244-8136

DOI: https://doi.org/10.53766/AcBio/2024.14.28.10

biology of chlamydiae. Microbes and infection. 2015; 17: 732-737.

[12] Goy G, Croxatto A, Posfay-Barbe K, Gervaix A, Greub G. Development of a real-time PCR for the specific detection of Waddlia chondrophila in clinical samples. European journal of clinical microbiology & infectious diseases. 2009; 28: 1483-1486.

[13] Eisen JA. The RecA protein as a model molecule for molecular systematic studies of bacteria: comparison of trees of RecAs and 16S rRNAs from the same species. Journal of molecular evolution. 1995; 41: 1105-1123.

[14] Thompson C, Thompson F, Vandemeulebroecke K, Hoste B, Dawyndt P, Swings J. Use of recA as an alternative phylogenetic marker in the family Vibrionaceae. International Journal of Systematic and Evolutionary Microbiology. 2004; 54: 919-924.

[15] Dilbeck P, Evermann J, Crawford T, Ward A, Leathers C, Holland C, et al. Isolation of a previously undescribed rickettsia from an aborted bovine fetus. Journal of clinical microbiology. 1990; 28: 814-816.

[16] Siemer J, Theile O, Larbi Y, Fasching PA, Danso K, Kreienberg R, et al. Chlamydia trachomatis infection as a risk factor for infertility among women in Ghana, West Africa. The American journal of tropical medicine and hygiene. 2008; 78: 323-327.

[17] Kebbi-Beghdadi C, Lienard J, Uyttebroeck F, Baud D, Riederer BM, Greub G. Identification of immunogenic proteins of Waddlia chondrophila. PLoS One. 2012; 7: e28605.

[18] Baud D, Goy G, Osterheld M-C, Croxatto A, Borel N, Vial Y, et al. Role of Waddlia chondrophila placental infection in miscarriage.

Received: 05/05/2024
Accepted: 06/02/2024

Volumen 14, N° 28. Julio/Diciembre 2024
Depósito Legal: PPI201102ME3815
ISSN: 2244-8136

DOI: https://doi.org/10.53766/AcBio/2024.14.28.10

Emerging infectious diseases. 2014; 20: 460.

[19] Baud D, Vulliemoz N, Ammerdorffer A, Gyger J, Greub G, Castella V, et al. Waddlia chondrophila, a Chlamydia-related bacterium, has a negative impact on human spermatozoa. Human Reproduction. 2018; 33: 3-10.

Received: 05/05/2024
Accepted: 06/02/2024