The incidence of Coxiella infection in Iraqi women with early pregnancy loss

Noor Hasan Ali*, Baydaa Hameed Abdullah*, wassan Abdulkarreem Abbas*, Mamdouh Mohamed Elbahnasawy**

*Department of clinical biochemistry, college of pharmacy, Mustansiriyah university, Baghdad,Iraq
** Military Medical Academy,Cairo, Egypt

Abstract :

Query fever is a bacterial disease resulting from Coxiella burnetii (CB) infection in animals. It is well known that small ruminants who get Q fever lose their babies. People think pregnant women may also have lousy gestation results if they get the infection or the infection comes back to life during pregnancy.

This study looks at how serologic results of Q fever and early pregnancy loss (EPL) are linked. In Baghdad, including Madinat Al-Imamain Al-Kadhmain Teaching Hospital and Abu Ghraib Hospital, multicenter case-control research was conducted from September to December 2022. A total of 90 women were enrolled in this investigation. Sixty clinically suffered from early pregnancy loss by having a nonviable intrauterine pregnancy. The other 30 women were subjects of comparable age, the week of pregnancy, and the healthy subjects as controls. Using a commercial enzyme-linked immunosorbent assay (ELISA), serum samples were screened for antibodies against CB. Three (5%) cases tested positive for CB by ELISA. The study reveals no association between adverse gestation outcomes and positive Q fever serology. Age and abortion history were not significantly correlated with C. burnetii seropositivity, according to the findings of CB cases that tested positive results.

Keywords: Query fever; Coxiella burnetii; Early pregnancy loss; Enzyme-linked immunosorbent assay; Iraq

نسبة الإصابة بعدوى الكوكسيلا لدى النساء العراقيات اللواتي يعانين فقدان الحمل المبكر

نور حسن علي*، بيداء حميد عبد الله**، وسن عبد الكريم عباس*، ممدوح محمد البهنساوي**
*قسم العلوم المختبريه السريريه في كليه الصيدلة الجامعه المستنصرية
** الاكاديمية الطبية العسكرية، القاهرة، مصر

الخلاصة:

حمى الاستعلام Query fever هي مرض بكتيري ناتج عن عدوى بكتريا الكوكسيلا بورنيتي Coxiella burneti في الحيوانات. من المعروف أن المجترات الصغيرة التي تصاب بهذه الحمى تفقد صغارها. هنالك اعتقاد سائد أن النساء الحوامل قد يعانين أيضًا من نتائج حمل رديئة إذا أصبن بهذه العدوى أو عادت العدوى إلى الحياة أثناء الحمل. هذا العمل تضمن دراسة العلاقة بين نتائج حمى الاستعلام وفقدان الحمل المبكر لدى النساء. وقد تمت في بغداد في كل من مستشفى الامامين الكاظمين التعليمي ومستشفى أبو غريب للفترة من شهر أيلول 2022 الى شهر كانون الأول 2022. من مجموع 90 امراة شملتهم الدراسة 60 امراة منهم كان يعانين من فقدان المبكر للحمل اما النساء الثلاثون الاخريات موضوعات ذات عمر مماثل ونفس اسابيع الحمل وبحالة صحية جيدة وذلك للمقارنة. في هذه الدراسه تم استخدام تحليل الاليزا للتحري

عن مستضدات بكتريا الكوكزيلا في عينات مصل الدم بطريقة الاليزاز. اظهرت النتائج ان 3 حالات (5%) ايجابية بين النساء المصابات بفقدان المبكر للحمل. تكشف الدراسة عن عدم وجود علاقة بين نتائج الحمل السلبية والأمصال الإيجابية لحمى كيو. لم يكن العمر والإجهاض مرتبطين ارتباطًا وثيقًا بالتفاعلية المصليّة من بكتريا الكوكسيلا، وفقًا للنتائج الايجابية لحالات الاصابة.

الكلمات المفتاحية: حمى الاستعلام، بكتريا كوكزيلا برونيتي، فقدان الحمل المبكر، فحص الالايزا

Introduction

Coxiella burnetii (CB) is a Gram-negative, pleomorphic, strictly intracellular, 0.4–1.0m and 0.2–0.5m long and wide bacterium. It is classified as Bacteria, Proteobacteria, Gammaproteobacteria, Legionellales, Coxiellaceae, Coxiella, genus Coxiella, and species CB (1–3).

Humans can incubate CB for 14-28 days or longer depending on the inoculation dose, infection method, and antigenic phase. This pathogen's cell wall demands a structurally and antigenically unique LPS molecule (3). Based on structure, LPS contains Phase II and Phase I antigenic variants. Infected hosts' virulent CB Phase II features a shortened LPS molecule without an O-antigen. Phase-I fully virulent LPS. Phase II involves multiple embryonated egg or cell culture passages. Chromosomal deletions in a 38-kb CB genomic region produce LPS antigenic phase variation (4). Two morphological varieties of CB exist: the large cell variant (LCV) and the small cell variant (SCV), which may be seen under an electron microscope. SCV is little and dormant, while LCV is large and metabolically active. SCV resists stress of the environment and has a prolonged survival rate in harsh environments (4). CB infections, commonly known as coxiellosis in animals and Query fever in humans, are a major cause of abortions, decreased reproductive efficacy, and subclinical infections in ruminants (5).

Q fever is a global zoonosis caused by CB, a Rickettsiaceae intracellular bacterium. CB is phylogenetically separate from the rest of this family, as shown by molecular biology (6). In 1955, Q fever was documented in 51 countries on five continents; four decades later, the disease was reported in an additional eight nations. In addition, seroepidemiological surveys have revealed the presence of Q fever in some other nations (7). Q fever epidemiology varies widely. Most European countries neglect Q fever because to its rarity. Molecular epidemiology and evolution of this disease in ruminants need additional study. Genomic research will suggest outbreak causes. Pathogenesis of CB requires molecular studies. Pasteurizing dairy milk and improving awareness may prevent Q fever-zoonosis (8) (one of the treatments use for Q fever infection is Amoxicillin which contain 2- azetidinone compounds that acts as broad spectrum antibiotic against gm +ve and gm-ve) (9).

The nomenclature classifies pregnancy losses as elective abortion (10), pre-embryonic loss (11), early and late fetal death (12), recurrent pregnancy loss (13), ectopic pregnancy (14), , early pregnancy loss (15), and early and late fetal death (12). By utilizing the postmenstrual age, the gestational age can be determined. EPL is spontaneous loss of pregnancy prior to 13 weeks of gestation (16). Before clinical confirmation, many pregnancies are lost, 15% of clinically proven pregnancies end in spontaneous abortion, and 30% of conceptions result in live births (17). The European Society for Human Reproduction

Special Interest Group for Early Pregnancy defines early gestation loss differently to promote consistency (15). In Table 1, Reclassification of early pregnancy loss are summarized.

Table 1:Reclassification of early pregnancy loss (18).

Term	Definition
Pregnancy of unknown location (PUL)	Scan negative for pregnancy with positive hCG
Late pregnancy loss	Loss of FCA during a gestational age more than 12 weeks
Early pregnancy loss	Scan demonstrating an empty sac or a sac containing a fetus but no FCA (pregnancy lasting less than 12 weeks)
Biochemical pregnancy loss	Pregnancy not found by ultrasound
Fetal loss	Previous CRL measurement with the subsequent absence of fetal cardiac activity (FCA)
Empty sac	Ultrasound showing sac without any structure or minimal structures

When describing a miscarriage diagnosis in the medical record, use exact and suitable terminology. Current use has been criticized for obscurity and misunderstanding (19–21).

50–60% of EPL instances are due to conceptus chromosomal insufficiency, while up to 90% of cases with embryonic mortality early in gestation have an aberrant chromosome complement (22). Less than 10% of these disorders involve genetic or structural abnormalities (23). Unsurprisingly, older mothers had a five-to-seven-fold higher miscarriage risk (24).

The likelihood of abnormalities of numerical chromosomal occurring again is low, and 2% of early miscarriages result in a trisomy (25). However, a couple with repeated pregnancy losses and a normal conceptus karyotype is likely to miscarry (26–28). Other reasons for miscarriage infections (Trichomoniasis is one of infections that causes early pregnancy loss) (29), chemical agents, immunological factors, psychological factors, inherited conditions, trauma, and maternal illnesses.

Worldwide, 23 million miscarriages occur annually, 44 every minute (30).

Serology is the main laboratory test for Q fever. Serological assays use inactivated whole cells from phase I and phase II CB strains. Nine Mile phase I (RSA 493) is a CB reference strain (31). The present study aimed to assess the Q fever level of Iraqi women with early pregnancy loss, and the relationship between Q fever serologic results and (EPL) in a population of women with symptomatic pregnancy.

Patients and Methods

In Baghdad, including Madinat Al-Imamain Al-Kadhmain Teaching Hospital and Abu Ghraib Hospital, multicenter case-control research were conducted from September to December 2022. A total of 90 women were enrolled in this investigation. Sixty of them were clinically suffering from early pregnancy loss by having a nonviable intrauterine gestation that happens within rather 12–7 weeks of pregnancy and has either an empty gestational sac or one with an embryo or fetus inside. Still, no fetal cardiac activity (FCA) or has vaginal bleeding. The other 30 women were

subjects of comparable age, week pregnancy, and healthy subjects as controls.

Ethical Approval

The Mustansiriyh University College of Pharmacy's Ethical Committee gave its approval to this study. Before the agreement to participate in the study was recorded, all participants were made aware of its goals and expected advantages

Inclusion Criteria

Reproductive-age women from [18 to 38] years old for both patient and control women.

Exclusion Criteria

Women with chronic disease, such as: (Hypertension, Diabetic, Heart disease, Hyperthyroidism disease, Kidney disease, Arthritis, Stroke, Asthmatic patient, Autoimmune disease, Patients with toxoplasmosis, Patients with CMV, patient with mycoplasma infection, women of age below 18 years old, and above 38 years old.

Specimens

Five mL of venous blood specimen was obtained from each participant, which was placed in a gel tube and then left for an appropriate time (nearly 15-30 min) for allowing them to clot at 25°C followed by centrifugation at 4400 (rpm) for fifteen min. The obtained serum was divided into two tubes, the first one also divided into two groups for rapid detection of CMV and toxoplasma to exclude the sample and if the sample is free from these two pathogens the second part of serum was frozen below (-20) °C until it was used in the analysis of mycoplasma IgG, Coxiella IgG The analysis manner of mycoplasma IgG, Coxiella IgG. were summarized Figure 1.
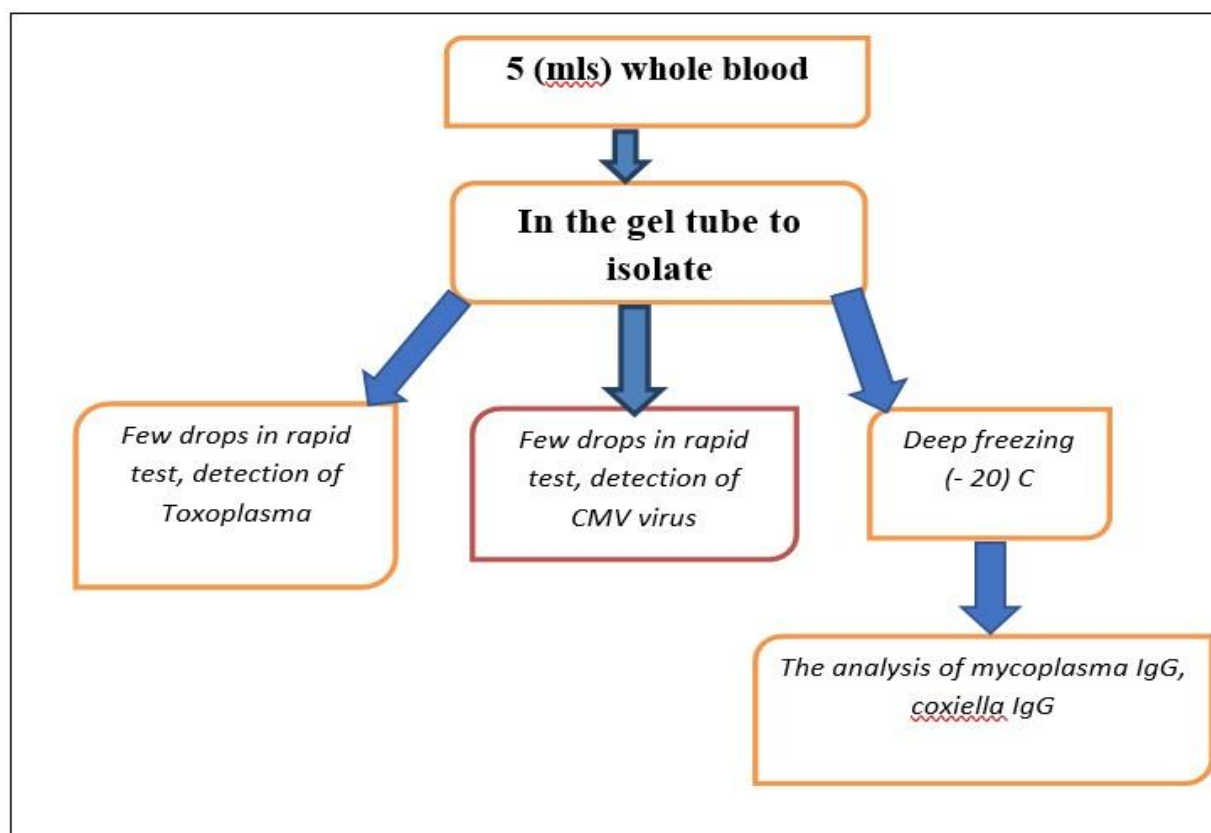

Figure 1: Specimen collection/analyzing procedure

Serological tests

It was opted for a one-step procedure to identify anti-CB antibodies. A commercial enzyme-linked immunosorbent assay (ELISA) was utilized to analyze each sample. The ELISA reagent was purchased from (MYBIOSOURCE, USA) and used following the manufacturer's instructions. Consider that some studies publish their findings based exclusively on ELISA when evaluating the relationship between spontaneous abortion and Q fever titers (32).

Statistical analysis

The V24 of SPSS software was used for statistical data evaluation. This study utilized descriptive analysis, which will provide 'summary' statistics such as mean, median and standard deviation. Descriptive statistics summarize facts and information numerically and graphically. Numerical techniques include measures of the central tendency and measures of variability. Descriptive analysis was used to examine variables by the group (t-test and Chi-square test). The ordinal logistic regression technique evaluated the association strength
between one dependent variable (number of pregnancy weeks) and independent variables (age, the group, coxiella burnetti). Goodness-of-Fit statistics were utilized for determining whether the model adequately describes the data (33).

RESULTS

The research population demographic characteristics are shown in Table 2. The women in both groups (patient= PW) and (control= CW) fell in the age group of 20–38 years old. Maternal age (by t-test) for women who experienced patient women PW presented at a significantly higher age than women (control) CW (the mean CW age:28.33 (yrs) 95% CI (26.42 – 30.25); the mean PW age: 28.82 (yrs) 95% confidence interval of mean (95%CI) 27.09 – 30.54 (P value:0.687) Figure 2a. For gestational age (by t-test), (mean CW gestational age:8.70 (weeks) 95% CI (7.41 – 9.99); mean PW gestational age: 9.47 (weeks) 95% CI (8.83 – 10.10) (P value:0.823) Figure 2b. For No. of children (by t-test), (mean PW children: 3.65 95% CI (2.99 – 4.31); mean CW No. of children: 2.80 (weeks) 95% CI (1.99 – 3.61). Among the groups, statistically, (P value:0.103), Figure 2c.

Table 2 compares the median Coxilla serum levels in PW versus CW. Serum Coxilla levels in PW were (median .9500, 95% CI (.8932– 1.0068) while the CW group Coxilla is constant when group = control and it has been omitted (P value:0.291) by Chi-square test. By indirect ELISA test after the addition of substrate to the wells any well get blue Color indicate positive presence of coxiella antigen. Both phase I and phase II antibody titers are often 1:128 or higher in Q fever samples. IgG class antibody titers occur early in the disease, peaking stage II by week 8 and lasting over a year. The number of positive and negative Coxilla analyses for the patient and control group were depicted in Figure 3.

Table 2: Descriptive analysis (examine variables by the group).

|	Group	N	Mean	95% confidence Interval of Mean		Median	Std. Deviation	Min.	Max.	Range	P value
				Lower Bound	Upper Bound						
Age	patients	60	28.82	27.09	30.54	28.50	6.668	20	38	18	0.687
	control	30	28.33	26.42	30.25	27.50	5.135	20	38	18	
No. of children (G)	patients	60	3.65	2.99	4.31	3.00	2.570	0	12	12	0.103
	control	30	2.8	1.99	3.61	3.00	2.156	0	8	8	
Week of Pregnancy	patients	60	9.47	8.83	10.10	10.00	2.446	4	13	9	0.823
	control	30	8.70	7.41	9.99	10.00	3.456	4	12	8	
Coxiella burnetii	patients	60	0.9500	0.8932	1.00681	1.00	0.21978	0.00	1.00	1.00	0.291
	Control[a]	30	[a] Coxilla burnetii is constant when group = control. It has been omitted								

(a)

(b)
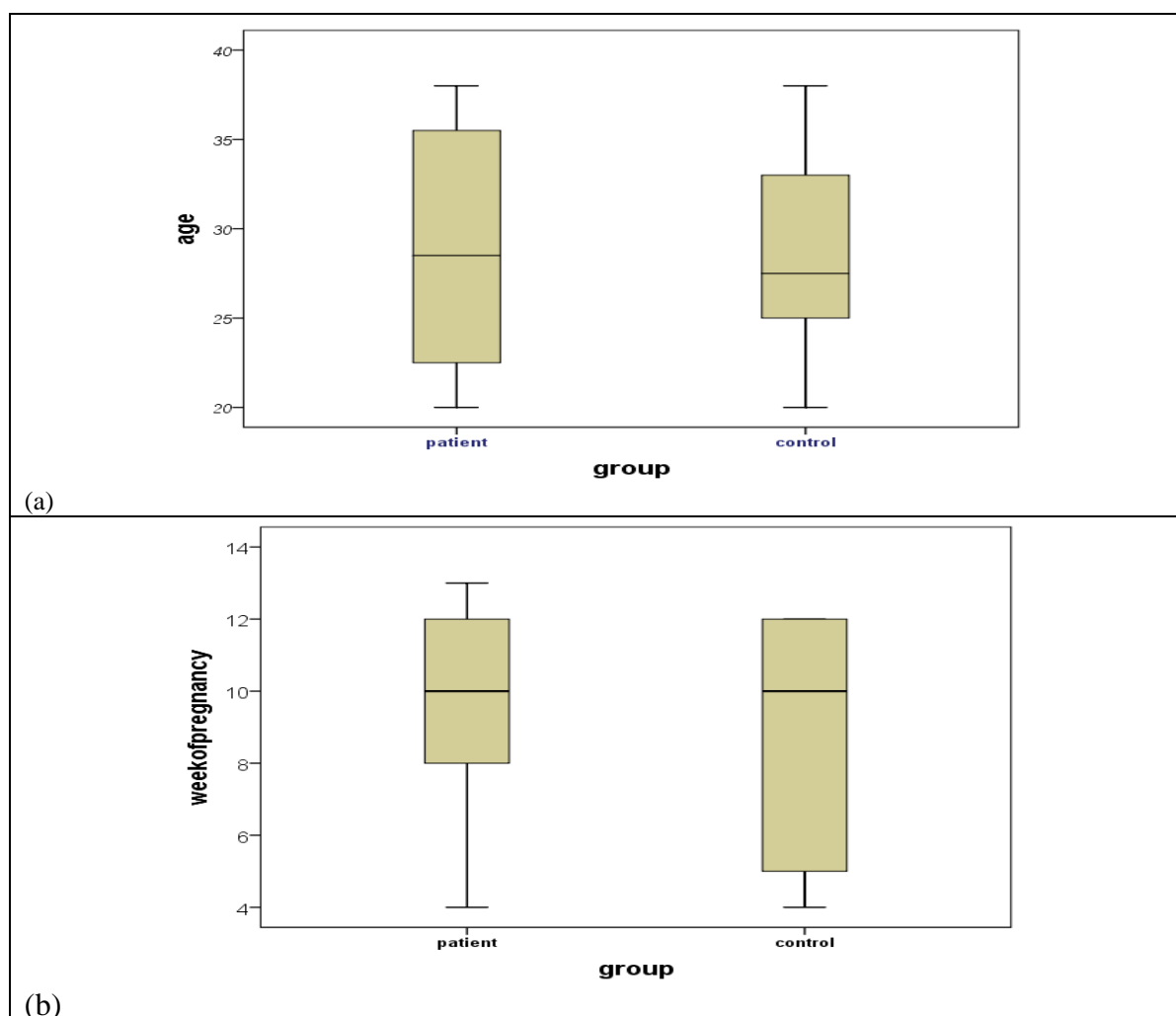
Figure 2. Stem-and-Leaf Plot for patient and control women (a) age (pvalue:0.687), (b) the week of pregnancy (pvalue:0.823), and (c) No. of the children (pvalue:0.103).
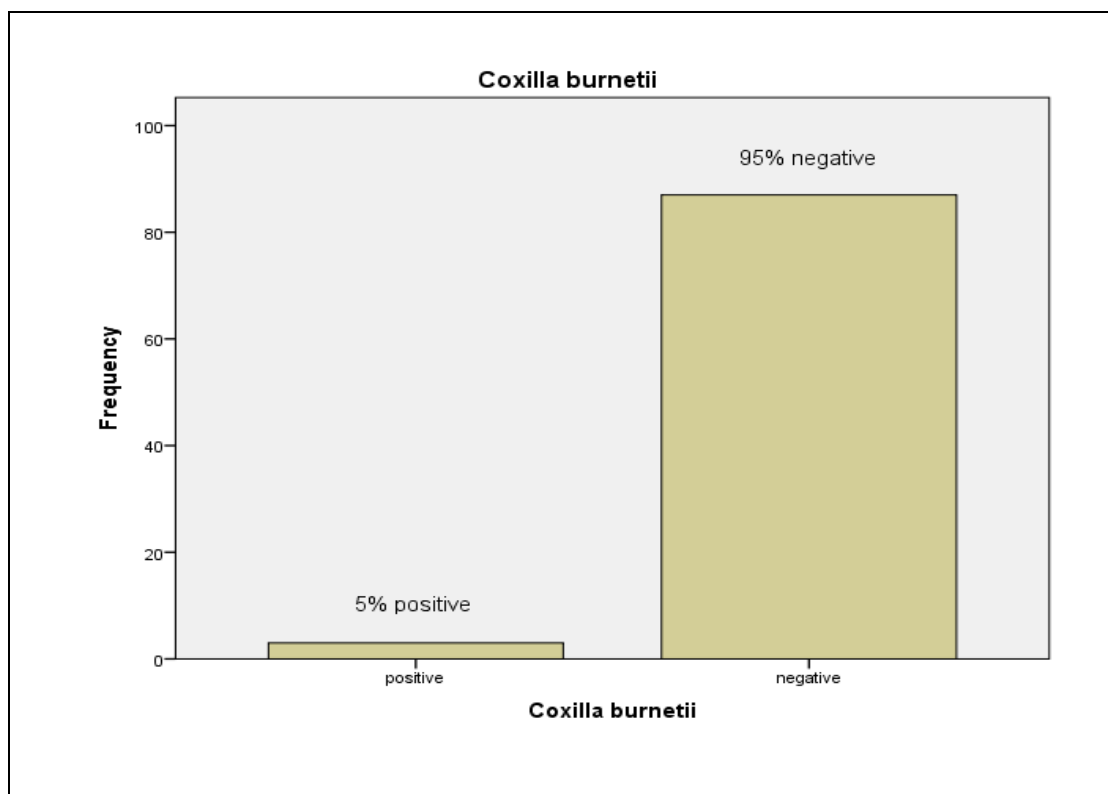
Figure 3. The positive and negative coxiella burnetii analysis.

The case processing summary is illustrated in Table 3, by using the ordinal logistic regression test; the higher marginal percentage of the week of pregnancy in this study was for 12, which was 37%, and the lower was 1.1% for the week of pregnancy of 13. The Marginal Percentage of the patient group was 66.7%, while for the control group was 33.3%. The positive Marginal Percentage was 3.3%, and the negative was 96.7%.

Table 3: Case Processing Summary (Ordinal logistic regression test)

		N	Marginal Percentage
week of pregnancy	4	4	4.4%
	5	12	13.3%
	6	7	7.8%
	8	14	15.6%
	9	6	6.7%
	10	8	8.9%
	11	4	4.4%
	12	34	37.8%
	13	1	1.1%
group	patient	60	66.7%
	control	30	33.3%
Coxillab	positive	3	5.0%
	negative	87	95.0%
Valid		90	100.0%
Missing		0	
Total		90	

Table 4: Model Fitting Information

Model	-2 Log Likelihood	Chi-Square	df	Sig.
Intercept Only	97.969			
Final	60.551	37.418	10	.000
Link function: Logit.				

Using goodness-of-fit statistics, one can determine whether or not a model adequately describes a data set Table 5. The significance value is displayed in the table (p > 0.05), indicating that the model satisfactorily fits the data. The term "goodness-of-fit test" is typically used to determine how closely the observed data conform to the presumed (fitting) model (34). In this instance, an insignificant finding would indicate that there are no significant deviations between the observed data and the model fitted to those data (measured).

Table 5. Goodness-of-Fit (for Ordinal logistic Regression Test)

	Chi-Square	df	Sig.
Pearson	246.594	269	.833
Deviance	170.910	269	1.000
Link function: Logit.			

The estimate, seen in Table 6, represents the probability of an instance falling into an associated category greater than the current category. The sign is understood to represent a linear regression. With the presence of a plus sign, the probability of an instance being assigned to a highly category in the variable of interest increases. The presence of a negative sign is linked to an increased risk of a case being assigned to a lower category in the dependent variable. For age, a positive indicator with age (0.044) would indicate that there is a higher relationship with pregnancy from 6-12 weeks and a lower relationship with pregnancy 4-5 weeks as the individual's age increases.

Table 6 Parameter Estimates (Ordinal logistic regression test)

		Estimate[1]	Std. Error	Wald	df	Sig.
Threshold	[wop = 4]	-1.606-	1.027	2.444	1	.118
	[wop = 5]	-.038-	.942	.002	1	.968
	[wop = 6]	.446	.938	.226	1	.635
	[wop = 8]	1.177	.943	1.556	1	.212
	[wop = 9]	1.451	.948	2.345	1	.126
	[wop= 10]	1.811	.954	3.600	1	.058
	[wop = 11]	1.996	.958	4.339	1	.037
	[wop = 12]	6.065	1.399	18.785	1	.000
Location	age	**.044**	**.031**	**2.026**	1	**.155**
	[group=0]	**.339**	**.403**	**.706**	1	**.401**
	[group=1]	**0ᵃ**	.	.	0	.
	[Cb.=.00]	**.485**	**1.096**	**.196**	1	**.658**
	[Cb.=1.00]	**0ᵃ**	.	.	0	.
Link function: Logit. Wop= weekofpregnancy. Cb.=coxiella b.						
1. OR = EXP(Estimate) will be used in next table.						
a. This value is set to 0 as it is unnecessary.						

For the patient group, for age, a positive sign with the patient group (0.339) would be that patient there is a higher (relationship) with pregnancy from (6-12) weeks and lower for pregnancy 4-5 weeks. For coxilla b. (0.485) is the same for the patient. **Table (7)**, The variables from ordinary logistic regression analysis. The findings revealed that age (OR = 0.0.1196, 95%CI: -0.17–.0105), the group (patient) (OR = 0.9215, 95%CI: -0.451–1.129), coxilla b. (patients) (OR = 1.318, 95%CI: -1.664–2.634) were identified as potential risk factors during pregnancy periods from (4-12) weeks. The value of (OR) was calculated as illustrated in Table 5 above.

Table (7) Odds ratio. (for the ordinal logistic regression test)

Variable	Estimate	OR	Std. Error	95% C. I.	P value
age	.044	0.1196	.031	-.017 - .105	**.155**
[group=0]	.339	0.9215	.403	-.451 – 1.129	**.401**
[group=1]	0ª	.	.	.	.
[Cb.=.00]	.485	1.318	1.096	-1.664 – 2.634	**.658**
[Cb.=1.00]	0ª	.	.	.	.

Table 7 shows the probability of falling into a higher or lower dependent variable category for each value variation in the independent variable. These odds are represented as a ratio between the two variables. When odds ratio is greater than one, it indicates a greater likelihood of being placed in a higher category for each additional unit of the predictor. OR<1 implies that the odds of belonging to a higher category fall as the predictor increases by one unit. This is indicated by the odds decreasing as predictor increases.

The ROC curve with area under the curve (AUC) of these parameters were shown in Fig. 4. Its shown that AUC near 0.5 and 95% CI (0.44 – 0.650).
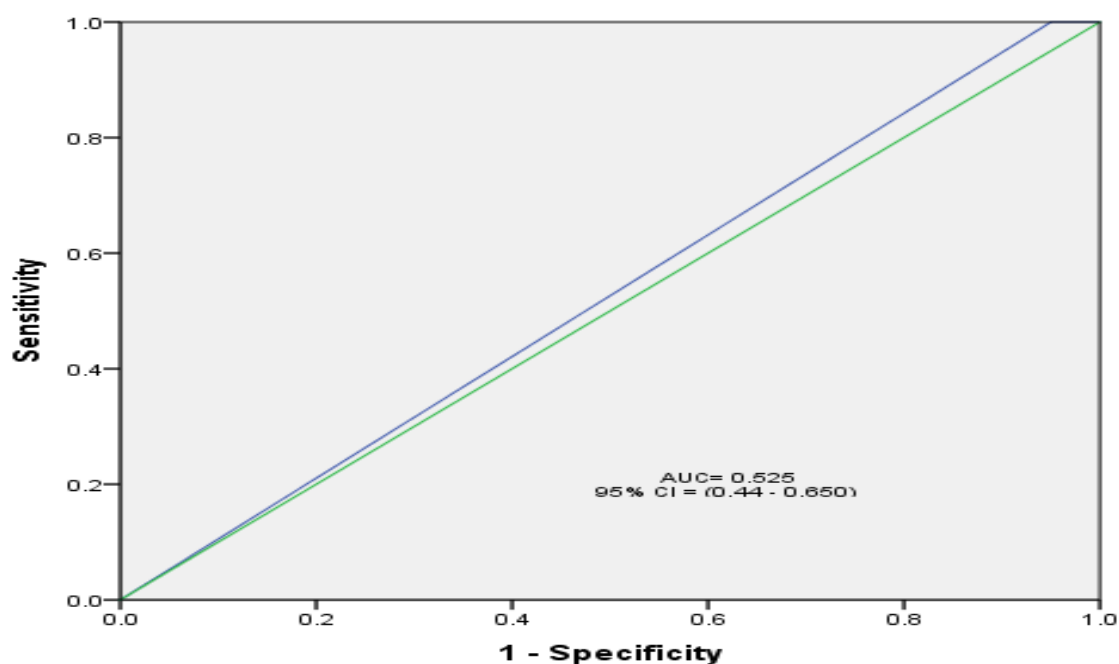
AUC= 0.525
95% CI = (0.44 - 0.650)

Figure 4: ROC curve analysis of the coxilla burnetii.

Discussion

The mounting evidence linking Q fever to bad gestation outcomes warrants screening in high-endemicity settings and treating infected mothers since Q fever is asymptomatic mainly (35). Some diseases enhance the likelihood of spontaneous abortions, although it's hard to prove (36). In this study, only three positive (Q-fever infection) cases could be classified as a probable chronic Q fever figure (2) ages 20-31 yrs old. This investigation found a week of pregnancy 9 -12 (week) from Abu Ghraib hospital. The mother's age is a significant factor affecting the probability of miscarriage (37). However, it is not reasonable to conclude that there is a correlation between age and the prevalence of C. burnetii infection (32). From the results, patient women (PW) presented at a significantly higher age than women (control) CW. Among the groups, there was a statistically considerable distinction in maternal age. It was found that there was a significant gestational age difference. Also, it was found that a significant number of children's differences existe

This study investigated Coxiella burnetii(CB) antibodies in serum samples using the ELISA assay instead of conventional serological techniques because the ELISA has greater sensitivity, is easier to execute in laboratories, is less expensive, and is quicker. Additionally, the ELISA test has a larger throughput, meaning more samples may be tested simultaneously (33) in addition to Wegdam-Blans et al. recommendation, they did not recommend phase I antibody detection for Q fever serological diagnosis (38).

Positive and negative results may not rule out current or past infections. The indirect diagnostic technique ELISA recognizes antibodies to detect CB exposure. Regional and national seroprevalence differences may be related to certain variables, including environmental circumstances, the time of research, and administration procedures, all of which may impact the transmission of C. burnetii (33). The ELISA test distinguished between the IgG and IgM classes of

antibodies to phase II antigen. The first blood antibody is IgM phase II, followed by IgG phase II. IgM and IgG phase II antibodies might last a year or more (32). As found by Jalel A. et al. investigation, that they reported Q. fever is an occupational disease, a zoonotic disease affecting people in the province of Thi-qar (Nasiriyah City-Iraq (39). Three cases in abugarib (this study) in the rural area and the exposure to the livestock leads to infection with Q. fever. Also, it could not link C. burnetii seropositivity to a poor pregnancy outcome with the same results by Stine Y. N. et al.(40), who studied implications and risks in pregnancies with rising or positive titers , suggesting that Denmark's Q fever risk –associated adverse gestation outcomes is low (19). Julien J. et al. conducted a serosurvey to determine pregnant women's Coxiella burnetii infection, for 203 pregnant women. They found that Q fever serum test were seropositive after seven months and seropositivity did not predict poor perinatal outcomes(41). Obaidat et al. evaluated Coxiella burneti seroprevalence and risk factors. They found that (the governorate of residency consuming of raw milk, sheep, goats, and dog ownership) strongly correlated with. This leads to what we found that rural area is C. burneti seropositivity source (42).

The true positive and negative rates are the main indicators of diagnostic accuracy. Specificity (the real negative rate) is the likelihood that the test accurately labels a healthy patient as negative. Sensitivity is the probability that the test accurately labels a diseased patient as positive (43). An AUC of 1 suggests a reliable test, while ≤ 0.5 cannot discriminate positive samples from negative controls (44) comparing with results found that AUC closed to 0.5 which cannot discriminate positive samples from negative controls.

Conclusion

According to the present study, pregnant Iraqi women with a history of animal contact were found to have Q fever. Based on the findings, pregnant women do not have a significant cause for concern regarding spontaneous abortions induced by coxiella burnetii. However, Q fever remains a risk factor for pregnant women. Concerning the direction of future investigation on spontaneous abortion and coxiella burnetii, it would be pertinent to examine adverse gestation outcomes in a population of pregnant women who are highly exposed, such as female laborers and veterinarians with contact with livestock. Coxiella burnetii should be considered for febrile disorders by Iraqi farmers and veterinarians. In conclusion, there was no relationship between spontaneous abortion and raised antibody titers against coxiella burnetii during the first trimester.

References:

1- Million M, Raoult D. Recent advances in the study of Q fever epidemiology , diagnosis and management. J Infect [Internet]. 2015;71:S2–9. Available from: http://dx.doi.org/10.1016/j.jinf.2015.04.024

2- Bielawska-dro A, Michalski A, Niemcewicz M, Bartoszcze M, Lasocki K. Prevalence of Coxiella burnetii in environmental samples collected from cattle farms in Eastern and Central Poland (2011 – 2012). 2014;174:600–6.

3- Abnae, Prasad et al. "Coxiella burnetii Lipopolysaccharide: What Do We Know?." International journal of molecular sciences vol. 18,12 2509. 23 Nov. 2017, doi:10.3390/ijms18122509.

4- Maltezou HC, Raoult D. Q fever in children. Lancet Infect Dis. 2002;2(11):686–91.

5- Plummer PJ, McClure JT, Menzies P, Morley PS, Van den Brom R, Van

Metre DC. Management of Coxiella burnetii infection in livestock populations and the associated zoonotic risk: A consensus statement. J Vet Intern Med. 2018;32(5):1481–94.

6- Rickettsies U, Public C. Phylogenic homogeneity of Coxiella burnetii strains as determinated by 16S ribosomal R N A sequencing. 1993;113:339–44.

7- Norlander, L. "Q fever epidemiology and pathogenesis." *Microbes and infection* vol. 2,4 (2000): 417-24. doi:10.1016/s1286-4579(00)00325-7

8- Ullah Q, Jamil T, Saqib M, Iqbal M, Neubauer H. Q Fever—A Neglected Zoonosis. *Microorganisms*. 2022;10(8): 1530. doi:10.3390/microorganisms10081530

9- AL-Tamimi EO, Muslih RM, Thejeel KA. Synthesis, Characterization and Antibacterial Studies of 2-azetidinones Compounds Derived from Amoxicillin. Al Mustansiriyah J Pharm Sci. 2015;15(1):14–23.

10- Smorti, Martina et al. "Centrality of Pregnancy and Prenatal Attachment in Pregnant Nulliparous After Recent Elective or Therapeutic Abortion." Frontiers in psychology vol. 11 607879. 3 Dec. 2020, doi:10.3389/fps.2020.607879.

11- C. N, W. A, S. D, A. KI, T. P, Z. G. M. Morphogenesis of extra-embryonic tissues directs the remodelling of the mouse embryo at implantation. Nat Commun [Internet]. 2019;10(1):1–12.

12- B. J, S. L, B.H. A, A. H, G. A, K. M. The placental vascular component in early and late intrauterine fetal death. Thromb Res [Internet]. 2012;130(6):901–5.

13- de Z. D, F. RF. Recurrent pregnancy losses, a lasting cause of infertility. Fertil Steril [Internet]. 2021;115(3):531–2.

14- K. G, G. I. E, H. S, C.HH, C. A, D. UP. Recurrent Tubal Ectopic Pregnancy Management and the Risk of a Third Ectopic Pregnancy. J Minim Invasive Gynecol . 2021;28(8):1497-1502.e1

15- P. MH, G. K, He M, K. S, S. R. Early Pregnancy Losses: Review of Nomenclature, Histopathology, and Possible Etiologies. FPP. 2018;37(3):191-209.

16- Amira MN Abdelrahman, BES. Serum Kisspeptin-1 at time of Pregnancy Diagnosis is superior to serum βhCG for prediction of Early Pregnancy Loss. Egypt J Fertil Steril. 2021;25(3):65–74.

17- M. NS, G. JPM, F. BCJM. Conception to ongoing pregnancy : the ` black box ' of early pregnancy loss. 2002;8(4):333–43.

18- M. S, G. B. Recurrent Pregnancy Loss. Springer Nature Singapore Pte Ltd. 2018.

19- Silver RM, Branch DW. Time for a Change. 2011;118(6):1402–8.

20- Condous G. Letter to the Editor Rede fi ning fi rst trimester miscarriage. 2012;597–8.

21- F. RG, J. E, E. N, S. E. Updated and revised nomenclature for description of early pregnancy events. 2005;20(11):3008–11.

22- E. RG. Causes of early embryonic loss in human pregnancy. 1986;1(3):185–98.

23- H. TJ. reviews Chromosome abnormalities in human reproductive wastage. Cell press journal. Vol 2, P105-110, 1986.

24- A. A N, W. J, C. P, O. J, M. M. Maternal age and fetal loss: population based register linkage study *BMJ* 2000; 320 :1708.

25- J.E, B. GJ. Pathophysiology of Histological Changes in Early Pregnancy Loss *. 2005;114–23.

26- O. M, A. K, O. S, S. K. Embryonic karyotype of abortuses in relation to the number of previous. 2000;73(2):300–4.

27- Predict A, Abortions S. Does the Karyotype of a Spontaneous Abortion Predict the Karyotype of a Subsequent Abortion？ -Evidence from 273 Women with Two Karyotyped Spontaneous Abortions. 1987;465–83.

28- S96 Abstracts,vol 76, No. 3, Suppl. 1, September 2001.

29- Dhilal M. and Hayat G. S. Prevalence trichomoniasis and candidiasis for symptomatic pregnant and non-pregnant women in Iraq. AJPS, Vol. 16, No.2. 2016.

30- Q. S, G. ID, D. S. RK, P. M, S.MD, F. J, et al. Miscarriage matters: the epidemiological, physical, psychological, and economic costs of early pregnancy loss. Lancet [Internet]. 2021;397(10285):1658–67.

31- M. HK, P. RA, K. GJ. Q Fever: A Troubling Disease and a Challenging Diagnosis.CMN. 2021;43(13):109–18.

32- S. C. M, G. EM, N. K, Knap JP. Prevalence of Coxiella burnetii Infection in Humans Occupationally Exposed to Animals in Poland. VBZD. 2015;15(4):261–7.

33- S. A, M. MA, A. A, A. FA, A. AH, B. HA, et al. Coxiella burnetii and its risk factors in cattle in Egypt: a seroepidemiological survey. BMC V.R. 2023;19(1):1–7.

34- Al-Khatib S, Al-Etan L, Abdo N, Al-Mistarehi A. Polymorphism in pro-inflammatory and immune-related genes in diffuse large B cell lymphoma susceptibility and overall survival in Arab descent population: A case-control study. Virchows Arch. 2019;475.

35- G. Z. N, P. M. Q fever during pregnancy: a narrative review. CMI [Internet]. 2020;26(7):864–70. Available from: https://doi.org/10.1016/j.cmi.2019.10.024

36- Nigro G, M. M, M. E, D. GC, C. G, A. MM. Role of the infections in recurrent spontaneous abortion. JMNM. 2011;24(8):983–9.

37- N. SY, H. NH, A. AMN, H. TB, K. B, K. KA, et al. Presence of antibodies against coxiella burnetii and risk of spontaneous abortion: A nested case-control study. PLoS One. 2012;7(2).

38- W. B. MCA, W. CCH, M. J, K.k JM, H. T, T. HT, et al. Evaluation of commonly used serological tests for detection of Coxiella burnetii antibodies in well-defined acute and follow-up sera. CVI. 2012;19(7):1110–5.

39- Jalel G, Alyaa H, A. Salih. Seroprevalence of Coxiella burnetii among humans in Nasiriya city- South of Iraq. JEPS 2010, vol. 1, Issue 1, P. 42-50.

40- N. SY, M. K, Villumsen S, H. TB, K. KA, L. CS. Adverse pregnancy outcomes and Coxiella burnetii antibodies in pregnant women, DEID. 2014;20(6):925–31.

41- J. J, Somon-Payet C, De V. P, Larrieu S Atiana L. Q fever seroprevalence in parturient women: The EQRUN cross-sectional study on Reunion Island. BMC Infect Dis. 2020;20(1):1–7.

42- 42. O. MM, Roess A, Imnadze P, Malania L, Salman AEB, Arner RJ. Seroprevalence and risk factors for coxiella burnetii in Jordan. Am J Trop Med Hyg. 2019;101(1):40–4.

43- Z. KH, Mauri L, O'Malley AJ,. Receiver-operating characteristic analysis for evaluating diagnostic tests and predictive models. Circulation. 2007;115(5):654–7.

44- Miller HK, Kersh GJ. Analysis of recombinant proteins for Q fever diagnostics. Sci Rep [Internet]. 2020;10(1):1–8. Available from: https://doi.org/10.1038/s41598-020-77343-0