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Sero-Detection of *Coxiella Burnett* (Q fever) Infection Anti-Antibodies Igg and Igm among Spontaneous, Recurrent Miscarriage Women in Gezira State, Sudan -Case-Control Studies 2018

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Abstract: ***Background*:** Q fever is a zoonosis caused by the bacterium *Coxiella burnetii* and is associated with epizootic abortion in ungulates. In humans, the role of Q fever during pregnancy has been recently questioned. ***Objectives:*** Thus, the goal of this study was to investigate the seroprevalence of *C. Burnett* infection among pregnant women in Gezira state, Sudan as well as the relationship between spontaneous recurrent abortion and pregnancy. ***Methods*:** This study included 90 women, half of whom had recurrent spontaneous abortion (RSA) and the other half had successful pregnancies. Blood samples were taken simply randomly, with ethical considerations, and using open ended closed questionnaire, prepared samples, and kept at -20°C. The seroprevalence of IgG and IgM antibodies against *C. burnetti* was determined using a commercial enzyme-linked immunosorbent assay test kit. Means of frequencies plus standard error, $Chi^{2,}$ and in depending sample t-test and logistic regression univariate and multivariate tests have been used. ***Result*:** The overall prevalence of *C. burnetii* in the study population sera was (37/90) 41.11 percent, with 5(11.1%) and 11(24.4 %) positive miscarriages in pregnant women for *C. Burnetti* IgM and IgG, respectively. While in nonmiscarriage were 8 (17.8 %) and 13 (28.9 %) positive for IgM (OR .578, CI-95% .174-1.926, P .372), and IgG (OR .796, CI-95% .312-2.032, P .634), respectively. Other significant risk factors detected in the study were age(OR 5, CI-95% 2-13, P 0.001), biomax index (OR 5, CI-95% 2-12, P 0.001), Diabetic patients (OR 11,1CI-95% 11 -11.38 P 0.000), disorder of thyroid (OR 8,9 CI-95% 8.1 -9 P 0.000), microcytic hypochromic anemia (OR 11, CI-95% 1.1 -110.2 P 0.04), Menstruation Cycle (OR 3.8, CI-95% 1.2 -11.5, P 0.02), vaginal bleeding(OR 6.4 CI-95% 2.1-19.2, P 0.001), pre-eclampsia(OR 16.1, CI-95% 1.9-131.1, P 0.01), and miscarriage family history(OR 2.9, CI-95% 2.8 -9.8, P 0.000), RDWC(OR 4.4, CI-95% 1.8 -10.9, P 0.002), RDWSD (OR 19.2 CI-95% 5.2 -71.1, P 0.000). ***Conclusion*:** The study concluded that the seroprevalence of *Coxiella burnetii* among miscarriage and nonpregnant women in Gezira State was dissociation with miscarriage. While selection risk factors associated with miscarriage include age, biomaxindex, Diabetic patients, a disorder of the thyroid, menstruation Cycle, vaginal bleeding, pre-eclampsia, miscarriage family history, microcytic hypochromic anemia, RDWC, and RDWSD.

Keywords: Q fever, *Coxiella Burnetii*, Miscarriage, Pregnancy, Sudan.

Research Paper

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INTRODUCTION

Q fever is a bacterial infection caused by *Coxiella burnetii*. It is typically a minor disease characterized by flu-like signs. Several people have no symptoms at all [1, 2]. *C. burnetii* can withstand harsh physical conditions, such as a hot and dry climate, for months or even years [1-3]. It survives in the environment in arthropod hosts such as ticks, and from these hosts, it can spread to domestic ruminants such as

animals, most notably sheep, goats, and cattle. People might be infected if inhale dust polluted by infected animals [1-4]. Raw milk and dairy products are a less widely reported route of transmission to Humans [5]. Although rare, person-to-person transmission has been documented and *C. burnetii* has been found in the sperm of infected males [6]. In humans, Q fever usually presents as asymptomatic or as a mild disease with spontaneous recovery. However, a small percentage of patients may undergo serious complications, including death [7]. With endocarditis, pneumonia, or hepatitis, illness might range from minor to severe. Mild symptoms include a high temperature, severe headache, muscle aches, disorientation, a scratchy throat, and a dry cough. Without any treatment, the majority of patients recover in a matter of months [8]. *C. burnetii* induces miscarriages in animals. Its consequences on human pregnancy and the treatment of Q fever during pregnancy are unknown [9, 10]. Recently published data from the Netherlands showed a prevalence of immunoglobulin IgM, suggesting recent infection with C. burnetii, in 3.4% of 1,646 tested serum samples from pregnant women in Q fever high-risk areas [11]. In Sudan, the exact prevalence of abortion is unknown; however, it was reported as 11% in the Safe Motherhood Survey 1999, and 9.9% in the Sudan Household Survey 2006 [12, 13] abortion in Sudan results in significant morbidity and mortality, because post-abortion care is not easily accessible [14]. *C. burnetii* infection has been reported to cause silent infections (asymptomatic) in communities which become endemic and could remain unnoticed for a very long time. In most parts of Sudan, these organisms are not screened for, and hence relative information about the frequencies of the organisms is sparse, so we conducted this case-control study to determine the prevalence of *C.burnetii* among Sudanese women.

MATERIAL AND METHOD

Between July and October 2018, a case-control study was conducted at the Teaching Hospital - Department of Obstetrics and Gynecology in Wad Madani city (the capital of Gezira state in east-central Sudan), Department of Obstetrics and Gynecology admits patients from rural areas. People who live in rural settings are frequently near animals, whether livestock or pets. This study included 90 women, half of whom had a recurrent spontaneous abortion (RSA) cases group and a control group who had successful pregnancies, blood samples were the sample size had an over 80% power to detect a difference of 5% at $\alpha = 0.05$; assuming a10% of the women might have incomplete data or samples. A volume of 5 ml blood specimen was collected from each patient through vein puncture technique, then displaced into plain containers, allowed to clot, centrifuged, and kept at -20°C until serological analysis. A complete blood count was performed using the hematological analyzer (Sysmex–XP 300). nd the Ethical Board of the Ministry of Health (Sudan). Data confidentially was maintained, and the information collected from all specimens had not been used for any purpose other than this study. Permission to investigate the specimens was granted by the director of Wad Madani Teaching Hospital (Al Gezira State, Sudan). Informed consent was obtained from patients before specimen collection direct questionnaire has been recorded about education, job, family history, vaginal bleeding, diabetes, hypertension, measles-mumps-rubella tetanus vaccines, blood group, and age were conducted in the hospital. The three main physical technology used were direct current impedance, advanced optical light scatter technology, and fluorescent flow cytometry and spectrophotometry, Using a commercial enzyme-linked immunosorbent assay test kit, the seroprevalence of IgG and IgM antibodies against *C. burnetti* were assessed ((indirect EUROIMMUNE Kit). For testing, the usage of controls, and the interpretation of results, the manufacturer's standard protocol was followed. The manufacturer's guidelines were used to determine the cutoff for a positive test result. According to the manufacturer's instructions, an antibody index [(sample OD/cut off serum mean OD) was calculated and interpreted as follows:

Calculating a ratio of the extinction value of the patient sample over the extinction value of the calibrator, and results are interpreted as follows:
Ratio <0.8: Negative
Ratio >0.8 to <1.1: borderline
Ratio >1.1: Positive

Data was computed and analyzed by using a statistical package for social science (SPSS) computer software version 20 program for interpretation of the result. The significance of the difference was determined using Means of frequencies plus standard error, $Chi^{2,}$ and in depending sample t-test and logistic regression univariate and multivariate tests have been used. which was employed to assess the association between variables; statistical significance was set at $p < 0.05$.

RESULTS

RBCs count very highly among the control group, packed cell volume (PCV) is slightly high in both cases and the mean of (RDW-CD) Red blood cell distribution width is 15.88 and (RDW-SD) red cell

distribution width measures the width size of red cell size distribution 52 .48 is high than normal range among the control group. The mean of other CBC parameters is within the normal range for both cases and control samples (Table 1).

Table 1: Clinical and Sociodemographic Traits of *C. BURNETT* Infection in case and Control Population

No	***Items***	***Control N=45 Mean* ± SEM**	**Case N=45 Mean ± SEM**	**P *value* (95% confidence interval)**
1	Age	26.02 ± 0.8531	30.89 ± 0.9504	0.0003 "-7.409 to -2.324"
2	Biomass index	25.66 ± 0.6089	27.85 ± 0.5751	0.0104 "-3.860 to -0.5250"
3	RBCs	10.65 ± 6.849	3.843 ± 0.1349	0.3235 "-6.836 to 20.44"
4	Hb	10.93 ± 0.2420	10.58 ± 0.3481	0.4187 "-0.4995 to 1.188"
5	TWBCs	9.109 ± 0.4661	7.907 ± 1.214	0.3577 "-1.386 to 3.790"
6	Platelets	251.7 ± 12.61	243.8 ± 14.61	0.6803 "-30.43 to 46.39"
7	PCV	33.94 ± 0.6871	31.84 ± 1.053	0.0984 "-0.4025 to 4.602"
8	MCV	90.72 ± 1.057	84.22 ± 1.010	0.0001 "3.590 to 9.410"
9	MCH	29.00 ± 0.5027	28.11 ± 0.5391	0.2311 "-0.5784 to 2.356"
10	MCHC	31.91 ± 0.3579	33.16 ± 0.3316	0.0125 "-2.216 to -0.2733"
11	MPV	8.687 ± 0.1015	9.593 ± 0.2327	0.0006 "-1.412 to -0.4012"
12	PCT	0.2115 ± 0.01113	0.2579 ± 0.03219	0.1762 "-0.1143 to 0.02136"
13	RDWCV	15.88 ± 0.2821	14.59 ± 0.3397	0.0044 "0.4121 to 2.170"
14	RWDSD	52.48 ± 0.8195	44.98 ± 0.8974	0.0001 "5.078 to 9.917"
15	Neutrophil	65.34 ± 1.864	66.43 ± 1.908	0.6829 "-6.403 to 4.216"
16	Lymphocyte	27.42 ± 1.617	32.19 ± 4.928	0.3599"-15.10 to 5.551"
17	Monocyte	4.627 ± 0.3153	5.324 ± 0.3098	0.1180 "-1.578 to 0.1822"
18	Eosinophil	2.553 ± 0.1767	2.267 ± 0.14	0.2108 "-0.1660 to 0.7394"
19	Basophil	00.00	00.00	Constant

RBCs (Red blood cells), Hb (Hemoglobin), PCV (Packed Cell Volume), MCV (Mean Cell Volume), MCH (Mean cell hemoglobin), TWBCs (Total White blood cells), MPV (Mean Platelet Volume), PCT (Plateletcrit), RDW-CV (Red Blood Cell Distribution Width), RDW-SD (Red Cell distribution width it measures the width of red cells size distribution.

ELISA was used to assess the sero-detection of *C. brunetti* IgG and IgM antibodies. *C. burnetii* was found in 41.11 percent of the study population's sera, with 5(11.1%) and 11 (24.4%) positive for *C. Burnett* IgM and IgG, respectively. IgM and IgG positivity rates were 8 (17.8%) and 13 (28.9%), respectively (Table 2).

Table 2: Evaluation of Elisa-Based Sero-Detection of *BRUNETTI* IGG and IGM Antibodies

Item	**Number**	**IgG**		**IgM**	
	Techniques	**ELISA**		**ELISA**	
		+ve	**-ve**	**+ve**	**-ve**
Miscarriage	45	11(24.4%)	34(75.6%)	5(11.1%)	40(88.9%)
No Miscarriage	45	13(28.9%)	32(71.1%)	8(17.8%)	37(82.2%)
Total	90	24(26.7%)	66(73.3%)	77	77(85.6%)

+ve: positive; -ve: negative

In this study, the findings suggest that *C. Burnett* does not directly contribute to the loss of spontaneous pregnancies. The biomass index and menstrual cycle factors were also investigated because they affect women's risk for miscarriage, preeclampsia, and vaginal bleeding. While tribes, jobs, education, miscarriage rate, MMR tetanus vaccine, hypertension, and blood group have no effect on miscarriage in our study population, thyroid, age, diabetes, and family history do in some cases (Table 3).

Table 3: Logistic Regression Analyses of the predictors for Miscarriage

		Univariate			Multivariate		
No	**Variable**	**OR**	**95% CI**	**P value**	**OR**	**95% CI**	**P value**
1	Tribes	0.93	.865-1.004	0.065	1.000	0.000-1.000	1.000
2	Education	1.107	.698-1.756	0.667	2.639	0.369-18.859	0.333
3	Jobs	.712	.420-1.206	0.206	1.000	0.000-1.000	1.000
4	Rate of Miscarriage	.000	0.000-0.000	0.993	1.000E-013	0.000-1.000	0.998734
5	Family history	2.94	2.946-948	.000	1.000	0.000-1.000	1.000
6	Menstruation Cycle	3.775	1.2-11.5	0.02	2.59	0.078-8.61	0.028
7	Vaginal disease	0.230	0.211-1.453	0.230	0.689	0.239-1.987	.491
8	Vaginal Bleeding	6.353	2.1-19.2	.001	1.39	.043-4.47	0.001
9	normochromic anemia	0.29	0.030-2.723	0.1	0.554	0170-1.801	.326
10	macrocytic anemia	2.1	0.723-5.846	0.176	0.554	0.170-1.801	.326
11	Microcytic hypochromic anemia	11	1.086-110.2	0.04	2.9	1.3-6.7	.000
12	Sero-positivity of Anti-Coxiella IgG*	.796	.312-2.032	.634	.820	.276-2.434	.721
13	Sero-positivity of Anti-CoxiellaIgM	.578	.174-1.926	.372	.821	.223-3.021	.767
16	MMR vaccine	0.389	0.130-1.166	0.1	3.919	0.758-20.268	0.103
17	Tetanus vaccine	9.649E8	0.000-1.166	1.1	1.7	.000-.000	0.997
18	all the vaccines MMR+TT	0.339.	0.109-1.058	0.1	3.375	0.845-13.473	.085
19	Diabetic patient	11.1	11-11.38	.000	10	0.10-10.3	0.476
20	Thyroid	8.9	8.1-8.9	000	8.739E-008	8.739E-8.739E-	0.476
21	Hypertension	1.08	0.065-17.8	0.96	0.972	0.057-15.741	0.951
22	Preeclampsia	16.1	1.9-131.1	0.01	2.983E-009	1.314E-010-6.776E-008	0.000
23	Blood group	0.000	.010-1.722	0.1	7.2	5.4-8.42	0.997
24	Age	5	2-13	0.001	.336	.090-1.250	.104
25	Biomass index	5	2-12	0.001	1.73	0.062-4.79	0.001
26	HB	1.3	0.56-3.1	0.4	0.574	0.126-2.615	.473
27	RBCs	2.3	0.85-6.2	0.1	0.494	0.156-1.564	0.230
28	Platelets	0.7	0.21-2.2	0.52	1.208	0.343-4.251	.768
29	TWBCS	0.7	0.254-1.97	0.5	1.928	0.635-5.855	.246
30	Vaccination	0.6	0.23-1.4	0.23	1.277	0.280-5.831	0.753
31	PCV	.432	0.162-1.157	.095	.594	0.180-1.959	.392
32	MCV	1.000	0.234-4.271	1.000	1.571	.327-7.549	.573
33	MCH	.577	0.248-1.343	.202	0.442	0.165-1.188	0.106
34	MCHC	1.545	0.616-3.878	.354	2.112	0.759-5.881	.152
35	MPV	.302	.058-1.587	.157	0.677	0.085-5.401	0.713
36	PCT	1.000	0.269-3.724	1.000	0.811	0.144-4.576	0.813
37	RDWC	4.375	1.750-10.9	.002	3.531	1.190-10.472	.023
38	RDWSD	19.158	5.158-71.1	.000	17.019	4.187-69.179	.000
39	Neutrophil	0.518	0.044-6.037	0.599	.309	.012-4.033	.309
40	Monocyte	2.098	0.364- 12.1	0.407	4.718	0.256-87.032	0.297
41	Eosinophil	1.400	0.295-6.651	0.672	0.633	0.043-9.426	0.740
42	Basophil	1.400	0.295-6.651	0.672	0.356	0.054-2.322	0.280
43	Lymphocyte	1.680	0.405-6.962	0.474	1.036	0.154-6.989	0.971

CI: Confidence interval; OR: Odd Ratio

DISCUSSION

The etiological agent of Q (query) fever, *Coxiella burnetii*, causes abortions in ruminants and is suspected of causing adverse pregnancy outcomes in women. Infection in pregnant women is associated with high fetal mortality and morbidity, and the mother is at high risk of developing chronic Q fever. In this study, the prevalence of *C. burentti* among pregnant women in the Department of Obstetrics and Gynecology in Wad Madani city (the capital of Gezira state in east-central Sudan) and the contribution of *C. burentti* infection to miscarriage was investigated. The results showed a high prevalence of *C. burentii*, approximately 41.11% among pregnant women, this result is lower than that a study done in France showed that 81% of the pregnant women with untreated Q fever had a miscarriage [15]. Another study done among veterinarians in Dernmak ranges from 36% to 47% [16]. Another study in Iran showed that 29.3%. All the previous studies mentioned above were higher in percentage than our research, and this may be due to the large sample size [16]. A prevalence of 29.3% was reported from pregnant women who had regular contact with farm animals in rural Iranian areas [11]. Being older, urbanity, low income, contact with animals, and tap water consumption were all identified as potential risk factors for Q fever. Age, urbanity, living situation, pregnancy status, abortion history, occupation,

and consumption of tap water were all positively correlated (p 0.05) with Q fever [11]. However, there was no clear cut-off point for the contribution of *C. burentti* infection to miscarriage, more detailed studies may be required to explain the actual impact of *C. burentii* infection in pregnant women. The study indicated that *Coxiella burnetii* infections common among younger women within the age group (18-28year) is (6.6%) and (29-39 years)is (7.8%), and this reflected a higher significant correlation with seropositivity (P=0.001), this result was in alignment with all studies that came to our knowledge done in the Netherlands [15-17]. They reported that the infection was common among younger women. This may be due to the type of epithelium predominant in younger women which is considered a target for *Coxiella burnetii.* Also, the results of this research showed that *Coxiella burnetii* infection was common among women with vaginal bleeding leading to an increased rate of miscarriage, contrary to studies in the Dutch [18]. These study findings revealed a difference in the percentage of infection between women with a history of abortion (8.8%) and without a history of abortion (5.5%) showed a statistically significant correlation with seropositivity (P =0. 000), contrary to the Dutch [18]. While in the Sudan they revealed its association with recurrent abortion, and they recorded that this may be due to reactivation of latent Coxiella burnetii infection or damage from past Coxiella infection.

WHO (2004) found that Coxiella burnetii infection is common among women with abortion with vaginal bleeding and they recorded that this may be due to Coxiella direct zygote infection [19]. The prevalence of antibodies against Coxiella burnetii was different in women with vaginal bleeding 8 (8.8%) and 5 (5.5%) in the women do not have vaginal bleeding times of previous pregnancies, showed statistically significant correlation with seropositivity (P =0. 001).

Both cases and controls have increased IgM and IgG seroprevalence, which may suggest infections might be caused by different strains. Finally, a recent study found that women who contract acute Q fever shortly before pregnancy do not have an increased risk of abortion or premature delivery. Ghanem-Zoubi and Paul [12], demonstrated that evidence suggests that Q fever increases the risk of early pregnancy abortion and late pregnancy prematurity or intrauterine fetal death. There have been studies from the Netherlands and Denmark that show no evidence of an increased risk of abortion among women, but they have some limitations such as study population and strain specificity [17]. Family history and age factors that contribute to miscarriage appear to be common among cases. Further studies with a large number of samples and more confirmatory advanced techniques are required to validate the results of the present study. Conclusions: Women in rural areas in Sudan frequently contract Q fever. To reduce unfavorable pregnancy outcomes, there is a need for an awareness program about the significance of *C. burnetii* infections and prevention strategies in women during pregnancy.

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