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Research Article

Prevalence of Brucellosis among Malaria Negative Febrile Participants by Real Time PCR in Jazan Region Southwest Saudi Arabia

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Abstract

Background: Southwest Saudi Arabia is known to be endemic with Brucellosis. The diagnosis of brucellosis is a challenge, culture of the organism from the bone marrow, blood and other tissues is the gold standard for diagnosis in brucellosis. However, it is invasive, time consuming and less sensitive, so the use of alternative methods for rapid and accurate diagnosis is an ultimate aim.

Purpose: To detect the occurrence of brucellosis among malaria negative febrile participants by real time PCR in Jazan region.

Methods: A total of one hundred and twenty members had been involved in this study from different Jazan provinces. 5 ml blood samples have been gathered from all individuals and examined for the presence and absence of Brucellosis causing bacteria DNA by Real time PCR.

Results: The overall occurrence of brucellosis detected by real time PCR among study population was 10%. Significant findings with p value < 0.05 were detected from Harub province (50%), compared with provinces, Jazan (16.6%), then Samttah, Al-Ardah, and Al-Raith with (8.3%), most of them were shepherd occupation (40%), compared with other occupational groups, 83% were male compared with female (Figure 4) and within the age range of 46 to 55 years old. No significance differences were detected between raw milk drinkers 10.4% and direct animal contact participant's 6.4% (p > 0.05).

Conclusion: Application of Real time PCR in diagnosis of Brucellosis could be of great value for detection of current infection and control the disease. Brucellosis may be one of the primary causes of tropical fiver in Jazan region amongst febrile malaria negative peoples. Occupation, age, ingesting of raw milk, and direct contact with animals, were fundamental threat elements of the infection.

Keywords: *Brucellosis; Real Time PCR, Southwest, Saudi Arabia*

Introduction

Brucellosis is a prime health situation problem [1,2]. In Saudi Arabia its high prevalence has been largely attributed to the social norms of the nomadic folks that live in close proximity with livestock and consume raw milk or milk product [3,4]. Southwest Saudi Ara-

bia is understood to be endemic with Brucellosis. Laboratory records imply that a sizeable share of the population within the southern region (19.2%) had serological evidence of exposure [5]. Massive studies conducted in southern Saudi Arabia over a long time [6,7] have documented and highlighted inside the trouble: nearly 60% of sufferers with extended fever had laboratory-documented brucellosis. *B. melitensis* became the most strain identified from those cases.

Indeed the place is wealthy in home animals and close contact with goats, sheep, cows and camels similarly to intake of raw milk were the main threat elements of acquiring the disorder. Benjamine [8] concluded that brucellosis is a common youth problem inside the south-west: maximum of the admitted instances (61.5%) were residents of Tihama low lands and most people (84.6%) belonged to Sheppard. the superiority of brucellosis among stay inventory is 17%. Alarmingly, about 92% of febrile adult patients were ignorant concerning the mode of the disorder transmission [7]. Recently a 13.6% sero-preprevalance rate in Jazan region was mentioned by Ageely., *et al* [9].

The gold standard for the analysis of brucellosis is isolation of *Brucella* bacteria, however, isolation of *Brucella* is time, and resources consuming; it requires bio-safety facility level III and extraordinarily skilled technical personnel to deal with samples and live bacteria for eventual identity and biotyping. So, strategies based totally on the polymerase chain reaction (PCR) are becoming very useful and massive progress has been made lately to improve their sensitivity, specificity, and technical ease and to decrease costs [10].

Real-time PCR has recently been evolved [11-13]. The fundamental benefits of this approach are that it is able to be done in a very short time, does not require electrophoretic visualization, and avoids infection. The method may be used for the analysis of human brucellosis and discriminated among inactive, seropositive, and active states whilst it modified into used to check serum samples for which scientific findings were recognized [13]. There has been three separate Real-time PCRs have been advanced to mainly come to be aware about *B. abortus*, *B. melitensis* and *B. suis* on the species level [11].

Most of laboratory information knowledge approximately Brucellosis in Jazan region become rely on antibody detection, which became no longer differentiate between current and post infection, so the use of PCR emerge as an ultimate goal of this study to hit upon the occurrence of brucellosis among malaria negative febrile patients by Real time PCR.

Materials and Methods

Ethical consideration

This study was approved by scientific research ethics committee at Jazan University reference number REC40/1-017. Written consent was obtained from all participants.

Participants of the study

A total of one hundred twenty members had been involved in this study from different Jazan provinces, the study included a confirmed malaria negative febrile patients, some of them had direct contact with animal and/or consume raw milk product showed by means of questionnaire. Febrile malaria positive patients were excluded.

Samples

5 ml blood samples have been gathered from all individuals in EDTA tube, turned into examined for the presence and absence of Brucellosis causing bacteria DNA by Real time PCR.

DNA extraction

DNA was extracted from blood sample by using QIAamp DNA Mini kit (Qiagen, Germany) following manufacture instructions.

Real time PCR

A qualitative assay for *in vitro* diagnostics Real time PCR amplification kit: fast tract diagnostic (FTD) was used to detect Brucella bacteria species DNA in blood samples. Extracted DNA was examined by using FTD Kit (FTD-39-32, Luxembourg) following the manufacture

descriptions, with minor amendment. All DNA samples was run the usage of Applied Biosystems 7300 real-Time PCR machine (Applied Biosystems, USA). Plate setup, amplification methods, thermal profile, negative and positive controls, software analysis as noted by kit supplier. Positive samples appear when produced Cycle Threshold (Ct) up to 33, as compared with positive control (Brucella plasmid DNA), were as negative samples appear when there was no Ct or late after Ct 35 compared with negative control (Figure 1).

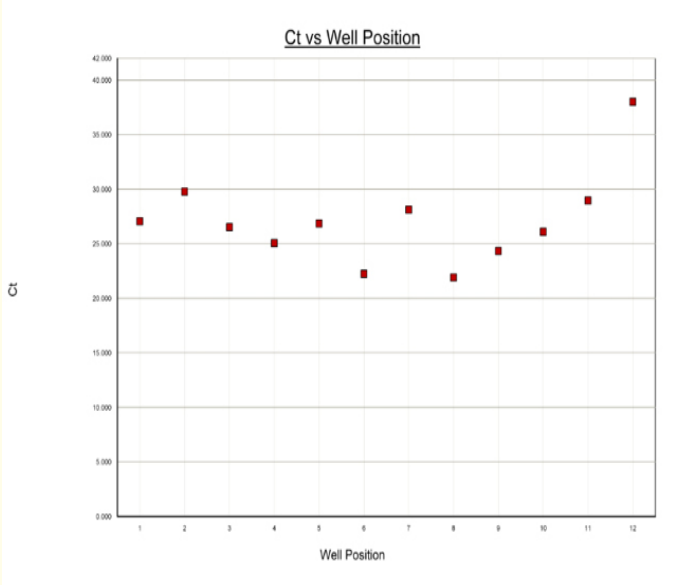

Figure 1: *Real time PCR graph Show wells: 1 to 10 a positive samples of Brucella DNA with (Ct) up to 30, well 11 positive control plasmid DNA with 29 Ct, and well 12 negative control with 38 Ct.*

Statistical analysis

Graphpad Prism (GraphPad Software, USA) was used for analysis. Values were measured as mean and median ± SD. P value less than 0.05 considered significant.

Results

The demographic statistics of individuals become proven on table 1. The overall occurrence of brucellosis detected by real time PCR among study population was 10%. Significant brucellosis cases were detected from Harub province (50%) $p < 0.05$, compared with others, Jazan (16.6%), then Samttah, Al-Ardah, and Al-Raith with (8.3%) (Figure 2), most of them were shepherd occupation (40%) $p < 0.05$, compared with other occupational groups (Figure 3), 83% were male $p < 0.05$ compared with female (Figure 4) and within the age range of 46 to 55 years old $p < 0.05$ (Figure 5). No significance differences were detected between raw milk drinkers 10.4% (5/48) and direct animal contact participant's 6.4% (2/31) $p > 0.05$.

Discussion

Most of laboratory records information approximately Brucellosis in Jazan region depend on antibody detection, which became no longer differentiate among current and past infections, so the usage of PCR to discover the bacterial DNA turn out to be an ultimate goal,

Characteristics	Number	Percentage %
Sex		
Male	91	75.8
Female	29	24.2
Age/year		
5 - 15	27	22.5
16 - 25	24	20.0
26 - 35	28	23.3
36 - 45	10	8.30
46 - 55	12	10.0
56 - 65	04	3.40
Not Stated	15	12.5
Provinces		
Harub	50	41.3
Samttah	24	21.5
AL-Ardah	25	20.3
AL-Eidabi	09	7.5
Jizan	07	5.3
AL-Raith	04	3.3
Fifa	01	0.8
Occupation		
Student	40	33.3
Shepard	12	10.0
Retired Officer	10	8.3
House Wife	20	16.6
Not working	13	10.8
Not Stated	25	20.8
Risk Factors		
Raw Milk Drinkers	48	40
Direct Animal Contact	31	26
None of Both Factors	21	17.5
Not Stated	20	16.5
Total	120	100

Table 1: *Demographic Data of study participants.*

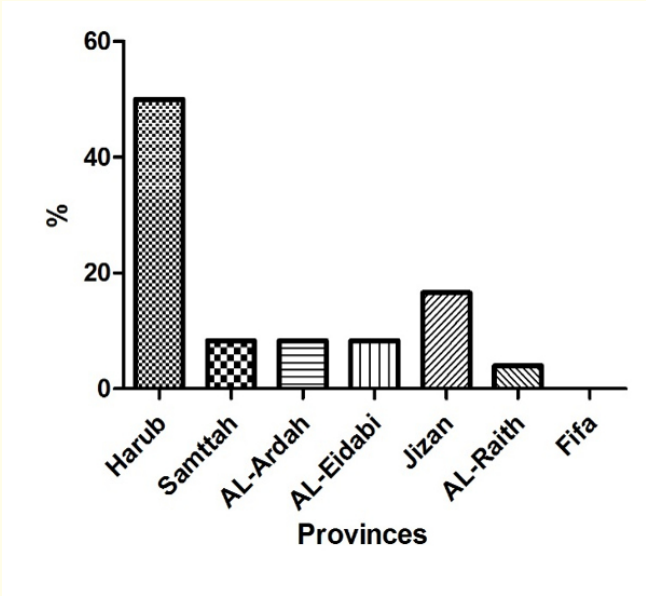

Figure 2: *Brucellosis result among different area (Provinces) of participants.*

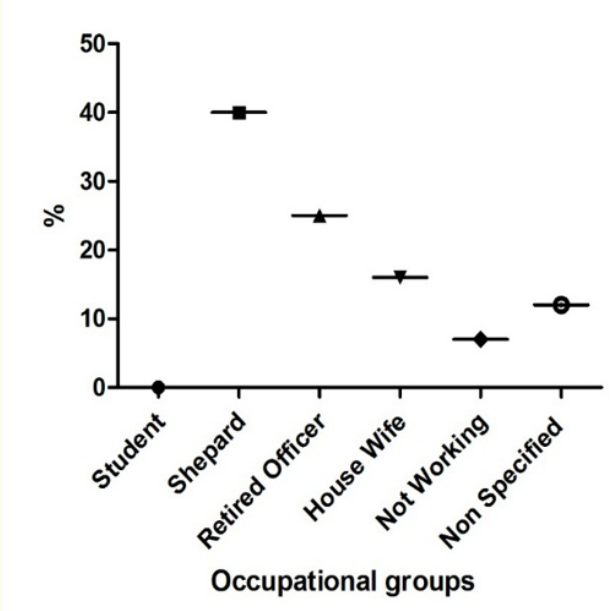

Figure 3: *Brucellosis results among occupational groups.*

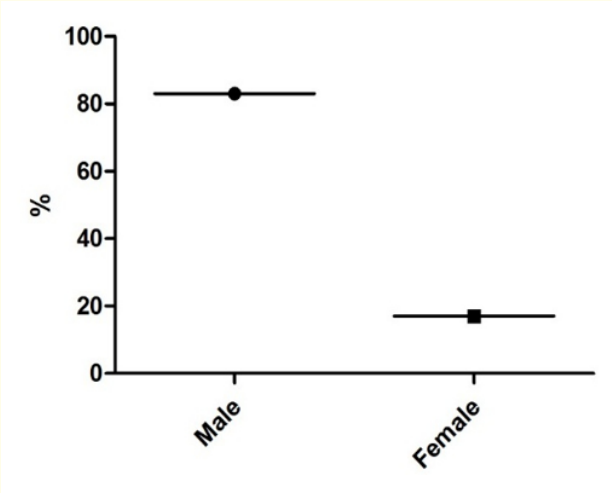

Figure 4: *Brucellosis results among Sex.*

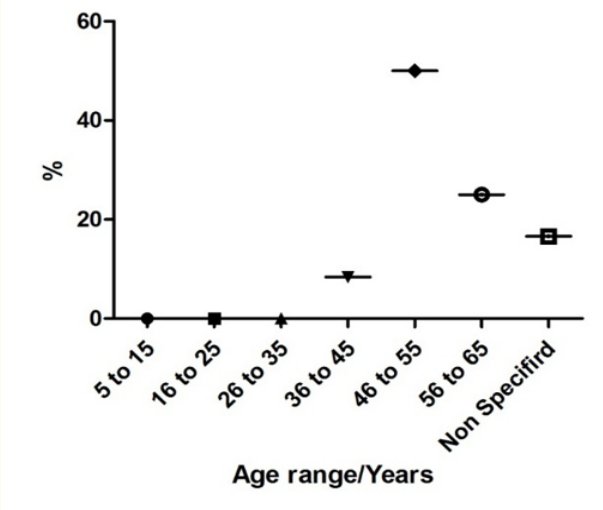

Figure 5: *Brucellosis results among age groups.*

as there has been difficulties to isolate the bacteria. This study was a primary record focused on detecting brucellosis by Real time PCR in Jazan area Southwest Saudi Arabia.

We found out that the general incidence of brucellosis in study population was 10%, indicated the presence of brucellosis can be one of the causes of tropical fiver in Jazan area. Our outcomes in settlement with examine conducted inside in the same place executed be Ageely., *et al*. [9], even though they used antibody detection technique. Remarkable similarity of our findings and different research accomplished

else were in Saudi Arabia [14]. Significant finding were found in men than women $p < 0.05\%$, which became a similar locating in lots of research in the country [15,16]. The better charge of brucellosis incidence in males compared with females became probably because of high exposure of men to the animals, and consumption of milk and milk products [9].

Substantial finding became detected among ages greater than 46 years old, in contrast with more youthful ages, this finding is consistent with observations made in neighboring Middle East countries [17,18]. The lower occurrence found in young adults, in evaluation with adults, may be because of greater publicity of adults to farm animals, and beside most of the young adults participating of this study were students.

Among occupational groups full-size most of brucellosis cases were detected among Shepard groups, compared with different groups, the findings explain that prolonged publicity and contact with animal can be a main threating component to get the infection. This end result changed into supported via examine carried out by Benjamine (1992) [8].

Even though there has been no significance differences among direct animal contact and dinking raw milk amongst study members, they nevertheless continue to be the chief threat factors.

Limitations

The most limitation of this study became based on small size of samples, so study include high population targeting both human and animals should be done in future.

Conclusion

Application of Real time PCR in diagnosis of Brucellosis could be of great value beside the routine other tests for detection of current infection and control the disease. Brucellosis may be one of the primary reasons of tropical fiver in Jazan region amongst febrile malaria negative peoples. Occupation, age, ingesting of raw milk, and direct contact with animals, were fundamental threat elements of the infection.

Acknowledgement

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Competing of Interest

The authors declare that there was no competing of interest of this study.

Bibliography

1. WHO, Brucellosis in humans and animals report (2006).
2. Abdelhady R., *et al*. "Prevalence of Brucellosis among Febrile Negative Malaria Patients by PCR in Northern Kordofan State, Sudan". *Journal of Clinical Microbiology* 6 (2017): 293.
3. Al-Sekait MA. "Epidemiology of brucellosis in Al medina region, Saudi Arabia". *Journal of Family and Community Medicine* 7.1 (2000): 47–53.
4. Memish Z and Mah M. "Brucellosis in laboratory workers at a Saudi Arabian Hospital". *American Journal of Infection Control* 29 (2001): 48-52.
5. Al-Balla SR. "Epidemiology of human brucellosis in southern Saudi Arabia". *The American Journal of Tropical Medicine and Hygiene* 98.3 (1995): 185-189.

6. Gaafar MM. "A clinical study of Brucellosis in adults in Aseer region of Southern Saudi Arabia". *American Society of Tropical Medicine and Hygiene* 56.4 (1997): 375-377.

7. Bilal N E., *et al*. "Study of Kindsy of attitude and practice (KAP) of Saudi Arabian community towards the problem of brucellosis". *Egyptian Journal of PHA* 66.1-2 (1991): 227-238.

8. Benjamin B and Annobil SH. "Childhood brucellosis in southwest Saudi Arabia. A five year experience". *Journal of Tropical Pediatrics* 38.4 (1992): 167-172.

9. Ageely., *et al*. "Prevalence and Risk Factors for Brucellosis in Jazan Province, Saudi Arabia". *Tropical Journal of Pharmaceutical Research* 15.1 (2016): 189-194.

10. Wei Ling Yu and Klaus Nielsen. "Molecular Detection and Typing of Brucella sp". *Croatian Medical Journal* 51 (2010): 306-313.

11. Redkar R., *et al*. "Real-time detection of Brucella abortus, Brucella melitensis and Brucella suis". *Molecular and Cellular Probes* 15 (2001): 43-52.

12. Queipo-Ortuno MI., *et al*. "Rapid diagnosis of human brucellosis by SYBR Green I-based real-time PCR assay and melting curve analysis in serum samples". *Clinical Microbiology and Infection* 11 (2005): 713-718.

13. Kattar MM., *et al*. "Development and evaluation of real-time polymerase chain reaction assays on whole blood and paraffin-embedded tissues for rapid diagnosis of human brucellosis". *Diagnostic Microbiology and Infectious Disease* 59 (2007): 23-32.

14. Elbeltagy KE. "An epidemiological profile of brucellosis in Tabuk Province, Saudi Arabia". *Eastern Mediterranean Health Journal* 7.4/5 (2001): 790-798.

15. Al Ali AM and Alluwaimi AM. "The Incidents of Human Brucellosis in Al-Ahsaa area, Saudi Arabia", *Scientific Journal of King Faisal University* 10.2 (2009): 1430.

16. Fallatah SM., *et al*. "Human brucellosis in Northern Saudi Arabia". *Saudi Medical Journal* 26 (2005): 1562-1566.

17. Araj GF and Azzam RA. "Seroprevalence of antibodies among persons in high-risk occupation in Lebanon". *Epidemiology and Infection* 117(1996): 281-288.

18. Lulu AR., *et al*. "Human brucellosis in Kuwait". *Quarterly Journal of Medicine* 66 (1998): 39-44.

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