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Coxiella burnetii Infection in Milk of Cattle and The Risk of Human Infection in Menoufia Governorate.

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ABSTRACT:

Q fever is a major anthropozoonotic disease infecting animals and man all over the world except in the Antarctic and New Zealand. This study aimed to highlight the public health significance of Q fever among dairy cattle and the potential role of raw milk products in transmitting the infection to humans in Menoufia governorate. A total of 60 raw milk samples were collected from 60 apparently healthy Baladi dairy cows of different ages from 3 localities in Menoufia governorate (20 from Shebein El-Kom, 20 from Tala and 20 from Menouf) and examined for presence of C. burnetii DNA by PCR. Results revealed that 25% (15/60) of milk samples had C. burnetii DNA. No significant association was revealed between PCR positivity and the cattle age or selected localities. Moreover, the current study involved estimation of seroprevalence of anti-C. burnetii phase II IgG antibodies by ELISA test among 184 attendants of Shebein El Kom fever and chest hospitals in Menoufia governorate in relation to the habit of consumption of unpasteurized milk products. 41.85% (77/184) of the examined individuals were positive for phase II IgG antibodies. A highly significant association was found between increased seroprevalence and the pattern of consumption of unpasteurized milk products per week since the patients with the habit of usual consumption (5-7 days/week) had the highest seroprevalence rate 56.10% (23/41) versus 47.14% (33/70) and 28.77% (21/73) among those with moderate (2-4 days/week) and rare (0-1 days/week) patterns of consumption, respectively. In conclusion, presence of C. burnetii DNA in milk of cattle and C. burnetii phase II IgG in contact human being could confirm the role of food from animal origin in transmission of Q fever to man.

Keywords: Coxiella burnetii, ELISA phase II IgG and PCR.

INTRODUCTION

Q fever is a globally distributed re-emerging bacterial disease that can be transmitted by several ways infecting humans, more than 30 species of domestic and wild animals, wild and domestic birds, reptiles, ticks and even marine mammals all over the world except in the Antarctic and New Zealand (Knobel et al., 2013). Coxiella burnetti (the cause of Q fever) is an obligate intracellular Gram negative, non-motile, non sporulated and pleomorphic microaerophilic (2.5%),

coccobacillus highly contagious bacterium (0.4 to 1µm length and 0.2 to 0.4 µm width) that can cause infection with a low infective dose (1 to 10 organisms), able to survive for long time in the environment and may be carried by wind to long distances (Heppell et al., 2017).

The primary main mode of Q fever transmission in humans is inhalation of pathogen-contaminated aerosols from excreta of infected goats, sheep and cattle especially birth products and even from excreta of infected parturient cats or dogs (Cetinkol et al., 2017). However, the risk of consumption of unpasteurized milk and milk products of dairy species (as cows, buffaloes, sheep, goats and camels) in transmission of Q fever to humans especially in the developed world countries couldn't be neglected (Pechstein et al., 2017).

In dairy cattle, although most C. burnetii infections are asymptomatic in nature, cattle may develop endometritis, infertility, abortion, sub-clinical mastitis resulting in reduction of milk production and final breakdown of the udder quarter (Johnson et al., 2019), the bacteria become localized in the mammary glands and continue to be shed in the milk for long periods of time up to 13 months so, the cow raw milk products were revealed to be positive for C. burnetii more frequently than that of goats or ewes since the excretion of C. burnetii can last only up to 52 days and 8 days in the milk of goats and sheep, respectively (Böttcher et al., 2011). Moreover, C. burnetii can survive more than 40 months in dry cow milk powder at room temperature, for over than 2 years and 8 years in skimmed milk kept at -20 °C and -65 °C, respectively (Sidi-Boumedine et al., 2010).

In humans, C. burnetii showed the ability for manipulation of the immune system and establishing a chronic infection after 6 months of infection persistence resulting in serious damage of person's vital organs (such as: heart, brain, liver and lungs). Spontaneous abortions and many adverse pregnancy outcomes also may develop among infected pregnant women (Rahaman et al., 2019). The prevention of Q fever among humans is mainly dependent on the prevention of the disease among animal populations. Furthermore, it is important to increase the public health awareness to avoid consumption of fresh milk. As well, proper pasteurization of milk at least 72 °C for 40 seconds is highly recommended (Gebremedhin and Shallom, 2018).

The aim of the current study was to perform examination of some raw cow milk samples in some localities in Menoufia Governorate by PCR technique to highlight their potential role as a risk factor in foodborne transmission of Q fever infection among examined humans attending Shebein El Kom fever and chest hospitals in Menoufia Governorate.

MATERIAL AND METHODS

Specimen collection:

A total of 60 milk samples were collected from 60 apparently healthy Baladi dairy cows of different ages from 3 localities in Menoufia Governorate (20 from Shebein El-Kom, 20 from Tala and 20 from Menouf). Teats of the cows were disinfected using a piece of cotton soaked in 70% ethanol and were let to dry with discarding the first few milliliters of foremilk. From each cow, fresh milk samples (30 ml each) were collected in clean plastic containers. The samples were frozen at -20 °C till examined for presence of C. burnetii DNA by using PCR technique. The cattle milk samples were randomly collected in the period from February 2018 to June 2018.

A total of 184 attendants of Shebein El Kom fever hospital and Shebein El Kom chest hospital (the main fever and chest hospitals in Menoufia Governorate) were selected for this study. By using sterile disposable syringes, approximately 5 ml of human blood were collected from the cephalic vein of each individual without anticoagulant. In a sterile glass tube, the blood was left to stand for about half an hour and then centrifuged at 3000 rpm for 10 minutes to obtain non haemolyzed clear serum that stored in labeled aliquots at -20 °C until tested for anti-C. burnetii phase II IgG antibodies by ELISA in relation to history of consumption of unpasteurized milk products (such as: Karish cheese and homemade butter) in the period from March 2018 to February 2019.

Detection of anti-C. burnetii phase II IgG antibodies by ELISA assay:

All collected human serum samples were examined for phase II IgG antibodies according to Samaha et al. (2012) by using the commercial indirect ELISA kit (Vircell SL® Granada, Spain. G1001, 96 tests).

Extraction of C. burnetii DNA:

The whole milk samples were centrifuged for 15 minutes at 5000 rpm at 10 °C. The milk fat and the liquid were discarded whereas the resulting milk pellets were washed twice by using PBS with centrifugation in each time for 5 minutes at 5000 rpm at 10 °C to eliminate milk inhibiting substances and increase recovery of the milk DNA. Finally, the supernatant was discarded and the remaining somatic cells were resuspended in 200 µl PBS as described by El-Mahallawy et al. (2016). DNA extraction from raw cow milk samples was performed in accordance with the manufacturer's instructions of G-spinTM total DNA extraction kit (cat. no. IBT-QMS-GT1704 (R01-2012-01), intron biotechnology, Seoul, Korea).

Amplification and detection of C. burnetii DNA:

The primers of (com1) gene were used according to El-Mahallawy et al. (2016) as follows: forward 5'-CCCTGCAATTGGAACGAAG-3' and reverse 5'-GTTCTGATAATTGGCCGTCGACA-3'. The final volume of PCR reaction was set up in a 25 µl. Each PCR tube contained 12.5 µl of master mix, 1 µl of each primer, 6.5 µl of distilled water and 4 µl of the extracted DNA. In a thermal cycler model (GS1, Thermal Cycler Base Unit GS00001, USA), the cycling conditions were performed as denaturation at 95 °C for 10 minutes followed by 35 cycles at 95 °C for 30 seconds, at 55 °C for 30 seconds and 72 °C for 1 minute for each cycle. The final cycle was followed by an extension step at 72 °C for 10 minutes. A 10 µl of each PCR product was subjected to gel electrophoresis in 1.5% agarose gel electrophoresis (cat. no. PG-40005, 100 g, Genetix Biotech Asia Pvt. Ltd., New Delhi, India) with using GeneDirex®100 bp DNA ladder H3 RTU (cat. no. DM003-R500, Focus Bioscience, Australia) as a marker. The expected PCR product size was 775 bp. Nine-Mile strain of C. burnetii which was used as positive control, was kindly obtained from department of molecular diagnostics and therapeutics in genetic engineering and biotechnology research institute (GEBRI), university of Sadat city (USC), Egypt.

Statistical analysis:

Data were analyzed using Chi- Square analysis test (X2) by using SPSS (Statistical Package for Social Science) version 17 by as was performed by Shabbir et al. (2016).

RESULTS:

Table (1): Molecular detection of *C. burnetii* DNA in the examined cattle milk samples (MS) by using PCR:

Test	No. of tested MS.	Positive		Negative	
		No.	%	No.	%
PCR	**60**	15	25	45	75

Table (2): PCR results in the examined cattle milk samples in relation to cattle age:

Age group of cows	Positive PCR		Negative PCR	
	No.	%	No.	%
≤ 5 years (No.= 47)	11	23.40	36	76.60
> 5 years (No.= 13)	4	30.77	9	69.23
Total (No.= 60)	**15**	**25**	**45**	**75**

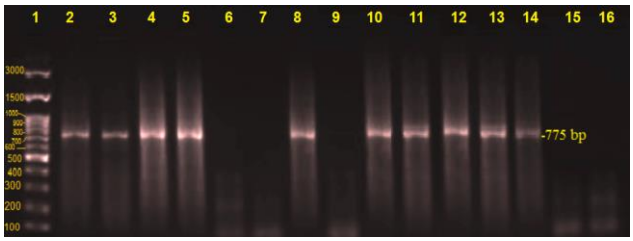

Figure (1): Ethidium bromide-stained agarose gel electrophoresis showing some representative results of PCR amplification of (*com1*) gene of *C. burnetii* in cattle milk. Lane 1: DNA ladder, lanes

2, 3, 4, 5, 10, 11, 12, 13 and 14: milk positive results, lanes 6, 7, 15 and 16: negative results, lane 9: negative control and lane 8: positive control.

Table (3): PCR results in the examined cattle milk samples in relation to different localities in Menoufia Governorate:

Localities of examined cows	Positive PCR		Negative PCR	
	No.	%	No.	%
Shebein El-Kom (No.=	6	30	14	70
Tala (No.= 20)	4	20	16	80
Menouf (No.= 20)	5	25	15	75
Total (No.= 60)	**15**	**25**	**45**	**75**
Chi- square	**0.533^{NS}**			
P-value	**766**			

Table (4): Seroprevalence of anti-*C. burnetii* phase II IgG antibodies in the tested patients in relation to consumption of unpasteurized milk products:

Pattern of consumption and No. of attendants who Consumed unpasteurized milk products (Karish cheese and homemade butter)	Positive phase II IgG		Negative phase II IgG	
	No.	%	No.	%
Rare (0-1 day/week) (No.= 73)	21	28.77	52	71.23
Moderate (2-4 days/week) (No.= 70)	33	47.14	37	52.86
Usual (5-7 days/week) (No.= 41)	23	56.10	18	43.90
Total (No.=184)	**77**	**41.85**	**107**	**58.15**
Chi- square	**9.36****			
P-value	**0.009**			

^{NS} Non Significant.

DISCUSSION

The result presented in table (1) revealed PCR positivity among raw cow milk samples was 25% (15 out of 60). The obtained result in cattle milk was in agreement with the findings of Guatteo et al. (2006) who illustrated a PCR positivity of 24.38% (59 out of 242) among cattle milk in France, Amin and Ahmed (2009) who showed a PCR positivity of 22% (22 out of 100) among cattle milk in Assiut Governorate, Egypt, Mohammed et al. (2014) who declared a PCR positivity of 28.95% (11 out of 38) among cattle milk in Saudi Arabia and Can et al. (2015) who detected a PCR positivity 24.38% (59 out of 242) among cattle milk in Turkey.

In contrary, some molecular studies on cattle milk revealed lower PCR positivity rates as: 4.74% (17 out of 359) in Switzerland (Fretz et al., 2007), 1.22% (3 out of 245) in France (Cardinale et al., 2014), 12% (18 out of 150) in Iran (Ahmadizadeh et al., 2015), 14% (7 out of 50) in Assiut Governorate, Egypt (Koriem et al., 2015), 1.43% (5 out of 350) in Turkey (Saglam and Sahin, 2016)

0% (0 out of 130) in Iran (Norouzian et al., 2018), 3.57% (4 out of 112) in Brazil (de Souza Ribeiro Mioni et al., 2019) and 5.53% (12 out of 217) in India (Dhaka et al., 2019).

On the other hand, higher PCR positivity rates were detected among cattle milk as: 94.30% (298 out of 316) in USA (Kim et al., 2005), 56.60% (193 out of 341) in the Netherlands (Muskens et al., 2011), 51.69% (92 out of 178) in Spain (Astobiza et al., 2012) and 48% (96 out of 200) in Turkey (Parin and Kaya, 2015).

Although worldwide observations revealed that Q fever human outbreaks were more frequently related to small ruminants than to cattle, the role of dairy cattle in transmission of Q fever to humans could not be neglected since the prevalence of Q fever was worldwide higher 20% among dairy cattle herds than among beef herds with the risk of shedding of C. burnetii in milk for several years and possibly lifelong without appearance of significant clinical signs on infected dairy cows (Astobiza et al., 2012). PCR is considered an accurate diagnostic tool for detecting presence of the pathogen gene mainly in the acute stages of the disease so, the obtained prevalence of C. burnetii among examined dairy cattle could spot the light on the risk of dairy cattle in Menoufia Governorate for maintenance of C. burnetii chain and transmission of Q fever infection.

Furthermore, the obtained prevalence could be referred to the fact of expelling of C. burntii through infected birth fluids at time of parturition and through feces at any time from onset of infection distributing the pathogen everywhere and since C. burnetii can persist for long time in the environment withstanding harsh environmental conditions like dryness, high temperature, UV light and even chemical disinfectants. Thus, once the area become contaminated with C. burnetii, it becomes very difficult to be decontaminated (Oyston and Davies, 2011). Moreover, dry manure beside the farm area could increase the risk of widespread of Q fever infection in seropositive farms as a result of open windy areas with high animal densities and high temperature.

Table (2) clarified that the PCR positivity was higher 30.77% (4 out of 13) among cattle of age group (> 5 years) in comparison with a PCR positivity of 23.40% (11 out of 47) among cattle of age group (≤ 5 years). The difference between the two groups was statistically non-significant. The obtained result of increased Q fever prevalence with increased cattle age in the current study was in agreement with the findings of McCaughey et al. (2010) who recorded the age group (> 4-8 years) to have the highest Q fever prevalence 7.78% (224 out of 2879) in comparison with a prevalence rate of 5.78% (84 out of 1454) among age group (> 2-4 years) and a prevalence rate of 1.77% (15 out of 849) among age group (0-2 years) through their epidemiological study on cattle in UK and Ruiz-Fons et al. (2010) who declared that prevalence of C. burnetii was higher 6.72% (35 out of 521) among adult cows of age group (> 3 years) than the prevalence rate 6.19% (6 out of 97) among heifers (< 3 years) in Spain.

In Egypt, Hussein (1993) illustrated a higher prevalence of 30.28% (76 out of 251) among cattle age group (> 3 years) versus 21.74% (20 out of 92) among cattle age group (< 3 years) in Assiut, Sohag and Quena Governorates. As well, El-Mahallawy (2012) showed a higher prevalence 10.71% (9 out of 84) among cattle of age group (> 3 years) versus 0% (0 out of 8) among age group (< 3 years) in Ismailia Governorate.

Furthermore, Seo et al. (2017) represented the increase of Q fever prevalence with cattle age in South Korea as shown 18.41% (51 out of 277) among age group (> 5 years) in comparison with 6.76% (24 out of 355) among age group of (3-5 years) and 1.92% (2 out of 104) among age group (< 3 years). Another study in South Korea performed by Seo et al. (2019) also revealed Q fever prevalence of 3.64% (2 out of 55) among cattle age group (> 3 years) in comparison with prevalence rates of 0.78% (2 out of 257) and 0.22% (1 out of 448) among age groups (2-3 years) and (< 2 years), respectively.

The obtained result of increasing C. burnetii with age of dairy cattle could be referred to the increased hormonal imbalance and the decreased immunity that occur among multigravida increasing the organism multiplication in the placenta in large numbers as well as increasing in age is mainly associated with increased risk of exposure to different sources of infection (infected animals, infected dust, infected water,

infected birth outcomes, infected excreta as: urine and feces, ticks, infected bull semen) during life (Garcia-Ispierto, 2010).

Table (3) revealed the highest PCR positivity rate 30% (6 out of 20) was detected among cattle milk samples collected from Shebein El Kom followed by a PCR prevalence rates of 25% (5 out of 20) and 20% (4 out of 20) among cattle milk samples collected from Menouf and Tala, respectively. There was no obvious significant statistical variation between different groups indicating that Q fever infection is endemic in all selected localities of Menoufia governorate in the present study. The low infective dose of C. burnetii (1-10 organisms) could indicate that one positive case could give an indication about presence of Q fever infection within whole the locality (Klemmer et al., 2018). The present study on dairy cattle was valuable only in that it provided evidence for the C. burnetii circulation among dairy cattle (even if they seemed apparently healthy) in the studied localities and also gave an indication about the possibility to contract Q fever among people who contacted with these cattle.

Regarding the human results, Table (4) in the current study revealed that the patients with the history of usual consumption of unpasteurized milk products (as: Karish cheese and homemade butter) (5-7 days/week) had the highest seroprevalence rate of anti-C. burnetii phase II IgG antibodies 56.10% (23 out of 41) in comparison with seroprevalence rates of 47.14% (33 out of 70) among patients with the habit of moderate consumption of unpasteurized milk products (2-4 days/week) and 28.77% (21 out of 73) among patients who rarely consumed unpasteurized milk products (0-1 day/week). The statistical analysis using chi-square revealed that the difference between groups was highly significant.

The obtained result in the current study was in agreement with the findings of El-Mahallawy (2012) who illustrated through her study in Ismailia Governorate, Egypt that the highest seroprevalence rate of anti-C. burnetii phase II IgG antibodies was 21.25% (17 out of 80) among humans with the habit of regular consumption of raw or unpasteurized milk and its products in comparison with seroprevalence rates of 15.69% (8 out of 51) and 8.16% (4 out of 49) among humans who consumed unpasteurized milk products sometimes and rarely, respectively.

Furthermore, Sun et al. (2016) reported that the seroprevalence rate of anti-C. burnetii phase II IgG antibodies was higher 43.21% (105 out of 243) among cattle farmers and farm residents with a history of consumption of raw cow milk in China versus a seroprevalence rate of 20.17% (24 out of 119) among those who hadn't. In addition, Park et al. (2018) declared that the seroprevalence rate of anti-C. burnetii phase II IgG antibodies was higher 13.46% (7 out of 52) among dairy cattle farmers who had the habit of consumption of raw cow milk in Korea versus a seroprevalence rate of 10.88% (127 out of 1,167) among those who didn't ever consume raw cow milk. As well, Mostafavi et al. (2019) showed that the Iranian humans who consumed unpasteurized milk and dairy products had a higher seroprevalence rate of anti-C. burnetii phase II IgG antibodies 37.43% (67 out of 179) in comparison with a seroprevalence rate of 28.18% (51 out of 181) among those who didn't.

On the contrary, Wardrop et al. (2016) found in their study on humans in Kenya that there was no evidence of a correlation between consumption of cattle milk and C. burnetii seropositivity among humans. They reported that 2.54% (52 out of 2,049) of overall examined humans were positive for anti-C. burnetii phase II IgG antibodies with less than 1% of the examined individuals had the habit of consumption of raw milk.

Increasing seroprevalence of anti-C. burnetii phase II IgG antibodies with increasing the consumption of raw or unpasteurized milk products would be referred to the fact that the bacterium C. burnetii becomes localized in the cattle mammary glands and continues to be expelled in cow milk in all phases of lactation (colostral phase, phases I, II and III) for long periods of time or even during lifetime and because C. burnetii has the ability to resist elevated temperature during routine pasteurization (at 63 °C for 30 minutes or even 72 °C for 15-20 seconds). The production of some types of cheese and butter that does not include heat treatment of milk at higher temperature (as Karish cheese and homemade butter) renders these milk products become a health hazard to humans who consume them as components of a

healthy diet since their possible contamination with the pathogenic C. burnetii (Radinović et al., 2013).

Conclusion

We can conclude that the risk of consumption of unpasteurized milk products of dairy cows in transmission of Q fever to humans in Menoufia Governorate couldn't be neglected. Discarding of infected milk and proper pasteurization of apparently normal milk are highly recommended steps to eliminate the risk of zoonotic spread of Q fever from animals to man.

Ethical Approval

All procedures performed in this study including collection of human blood samples and milk from animals were in accordance with the Egyptian ethical standards of the national research committee. All human subjects gave their consent for the collection of the blood samples, with the agreement that any identifying details of the individuals should not be published.

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