



Serologic and Molecular Investigation of Q Fever on Water Buffalo in Afyon

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SUMMARY

The water buffalo were usually grown for milk production and famous by-products, semi-hard cheese and especially cream clotting in Afyon. In this study, it was aimed to determine the risk of Q fever infection among the water buffalos, in Afyon. The collected 92 serum and 92 blood samples of female water buffalos were examined to investigate for the different stages of infection. A total of 92 serum samples was examined for the presence of *Coxiella burnetii* (*C. burnetii*) antibodies against phase I and phase II antigens by indirect ELISA. Eight (8.69 %) out of 92 serum samples were found to be antibody positive. Of the examined blood samples, none of them were found to harbor *C. burnetii* DNA.

Key Words: Q Fever, Water buffalo, ELISA, PCR

ÖZET

Afyon İlinde Mandalarda Q Humması'nın Serolojik ve Moleküler Araştırılması

Afyon'da manda, süt ve süt yan ürünlerinden peynir, özellikle de kaymak üretimi amacıyla yetiştirilmektedir. Bu çalışmada, Afyon ilinde mandalarda Q humması riskini araştırmak amaçlanmıştır. Dişi mandalardan toplanan 92 serum ve 92 kan örneği, enfeksiyonun farklı dönemlerini tespit etmek amacıyla incelendi. Toplam 92 serum örneğinde, *Coxiella burnetii* (*C. burnetii*) faz 1 ve faz 2 antijenlerine karşı antikor varlığı indirekt ELISA ile incelendi. Doksaniki örneğin 8'i (%8.69) antikor pozitif bulundu. İncelenen kan örneklerinde *C. burnetii* DNA'sına rastlanmadı.

Anahtar Kelimeler: Q Humması, Manda, ELISA, PCR

INTRODUCTION

Animals typically acquire *C. burnetii* via exposure to infected animals, by direct contact with parturition products and also by inhalation of infectious aerosols. Domestic farm animals are the major source of Q fever infection. It is known that *C. burnetii* may cause abortion in sheep and goats, but not in cows. Cows are often naturally infected but usually do not show clinical signs. Even if reproductive disorders and mastitis occur, abortion in cows are believed to be rare (To 1998). Due to frequently chronically infected, and not showing clinical signs, persistent shedding of *C. burnetii* via milk is a major problem in cows (Maurin and 1999; Porter *et al.* 2011).

The water buffalo has been raised in Turkey for centuries, originating from Indian migration (7th Century) (Sekerden *et al.* 1996b). Water buffalo husbandry concentrates in the Black Sea Region, North of Middle Anatolia, Thrace, Hatay, Mus, Kars, Diyarbakir, Afyon, Sivas. Buffaloes are raised for milk production only as source of income that does not

require any expenditure, i.e. in the areas that have natural feeding conditions. Frequently, the water buffaloes were reared with the cow herds. The water buffaloes raised for milk production and famous by-products, semi-hard cheese and especially cream clotting, in Afyon (Sekerden *et al.* 1996a; Sekerden *et al.* 1996b). The studies on Q fever for water buffaloes were restricted in the world as well in Turkey (Adesiyun and Cazabon 1996; Perugini *et al.* 2009).

In acute Q fever infections, antibodies against phase II antigens were observed in high rates. In chronic infections, antibody response developed against phase I in addition to phase II antigens due to phase variation of *C. burnetii* (Rodolakis 2006). Investigations on Q fever mostly depend on serological diagnosis (Rousset *et al.* 2011). If the serological tests were compared to determine either acute or chronic stage of the infection, CFT and FAT were declared to have some disadvantages. ELISA is advantageous due to capable of detecting both anti-phase I and anti-phase II antibodies compared to FAT. And also, ELISA is superior than CFT because of high sensitivity for determining all IgG

subclasses (Rousset *et al.* 2011). Serological response occurs in earliest 7-15 days. For a definitive diagnosis of early stages of acute Q fever, serological testing in combination with PCR was recommended (Vaidya *et al.* 2010).

In this study, it was aimed to investigate the different stages of the infection (acute and chronic) in water buffaloes, in Afyon. In order to investigate the presence of antibodies against *C. burnetii*, serum samples were examined by Indirect ELISA. Blood samples were checked for the presence of *C. burnetii* by touch-down PCR.

MATERIALS and METHODS

Samples: In 2011, total 92 serum and 92 blood samples of water buffalo were collected from the province of Afyon, Turkey. In Afyon, traditional buffalo breeding was done by the families, eg. three to five buffaloes owned by the family. All the water buffaloes were selected randomly and none of them has a recorded abortion history or reproductive disorder. All the blood samples were taken from the water buffaloes reared by the families which were sent to slaughterhouse. Blood samples collected in EDTA anticoagulant blood tubes for DNA extraction and serum tubes for serological examination. All the sera were separated 24 hours after sampling. All serum and blood samples were stored at -20 °C until tested.

ELISA: The sera were tested for the presence of specific antibodies directed to both phase I and phase II antigens of *C. burnetii* using ELISA CHECKIT Q-fever test (Idexx Laboratories, Broomfield, CO, USA) according to the manufacturer's instructions.

Positive control: DNA extracted from positive strain containing the gene coding phase II antigen was kindly obtained from Firat University, Faculty of Veterinary Medicine, Department of Microbiology and used as a positive control.

DNA Extraction: Blood samples were extracted by commercial DNA isolation kit (QIAamp DNA Mini Kit; Qiagen, Cat no:51104) according to the manufacturer's instructions. DNAs were stored at -20 °C until used.

Primers: Trans-1(Forward) and Trans-2(Reverse) primers, complementary to the *IS1111* fragment, a transposon-like repetitive region were used for the diagnosis of *C. burnetii* by touchdown PCR. Primers as previously described by Hoover *et al.* (1992) consisted of the following sequences: Trans-1 (Forward); 5'-TAT GTA TCC ACC GTA GCC AGT C-3' and Trans-2 (Reverse); 5'-CCC AAC AAC ACC TCC TTA TTC-3'. Expected amplicon size was 687 bp.

Touchdown PCR: Each reaction had a volume of 25 µl including, 22 µl reaction mixture containing 2.5 µl 10 X PCR buffer (without MgCl₂), 0.5 µl dNTP (10 mM), 1.5 µl MgCl₂ (25 mM), a 1 µl of each primer (10 pmol/µl), 0.25 µl Taq DNA polymerase (5 U/µl) (Fermantas; EP 402), 15.25 µl deionized water and 3 µl template. Cycling parameters were as follows: initial denaturation at 95 °C for 2 min followed by 5 cycles of denaturation at 94 °C for 30 sec, 66 to 61 °C (the temperature was decreased by 1 °C between consecutive steps) for 1 min, extension 72 °C for 1 min and final extension 72 °C for 10 min (Berri *et al.* 2000). Touchdown PCR was performed using Thermal Cycler (Arktik, ThermoScientific).

Agarose Gel Electrophoresis: PCR products were electrophoresed on a 1.5 % agarose gel in TBE buffer containing 0.5 µg/µl of ethidium bromide at 100 V for 45 min and visualized under UV light.

RESULTS

In this study, 92 serum and 92 blood samples of female water buffaloes collected from the province of Afyon, were examined. A total of 92 serum samples was examined for the presence of *C. burnetii* antibodies against phase I and phase II antigens by indirect ELISA. Eight (8.69 %) out of 92 serum samples were found to be antibody positive. Of the examined blood samples, none of them were found to harbor *C. burnetii* DNA.

DISCUSSION

The epidemiology of Q-fever in water buffalo (*Bubalus bubalis*) is largely unknown worldwide. There is limited Q fever studies carried out on water buffalo in the world. The prevalence of Q fever on water buffaloes was ranged from 0% to 16.66% (Adesiyun and Cazabon 1996; Galiero *et al.* 1996; Kalema-Zikusoka *et al.* 2005; Perugini *et al.* 2009; Vaidya *et al.* 2010). In a study conducted on 164 aborted Italian water buffalo (*Bubalus bubalis*) fetuses, 17.5% infection prevalence was declared to be determined by one-tube nested PCR with a highest infection rate in placenta, followed by liver and spleen (Perugini *et al.* 2009). Galiero *et al.* (1996) reported that 1.2% positivity was found of the examined 1012 serum samples. The prevalence of antibodies to *C. burnetii* of the examined sera of 1011 buffaloes in India was announced to be 16.02% by Yadav *et al.* (1979). Vaidya *et al.* (2010) declared the prevalence of Q fever in buffaloes as 16.66% in Italy. In 1997, in a study conducted in Uganda on 42 free-ranging African buffaloes, none of the buffaloes was found to be *C. burnetii* positive (Kalema-Zikusoka *et al.* 2005).

In Turkey, a few regional studies investigating seroprevalence of Q fever in human, sheep, cattle, and goat were implemented (Cetinkaya *et al.* 2000; Gozalan *et al.* 2005; Kilic *et al.* 2005; Dogru *et al.* 2010; Kennerman *et al.* 2010; Gazyagci *et al.* 2011; Arserim *et al.* 2011; Ozturk *et al.* 2012). And also, PCR-based diagnostic methods were used to investigate Q fever such as in blood, aborted fetuses, milk of goat, sheep, cattle (Ongor *et al.* 2004; Kirkan *et al.* 2008; Gunaydin *et al.* 2014; Gunaydin *et al.* 2015). To our knowledge, there is hardly any study conducted on water buffalo on the subject of Q fever in Turkey. In 1952, in Ankara, the results of WHO survey showed that 2 out of 49 buffaloes were *C. burnetii* positive (Kaplan and Bertagna, 1955). Although we encountered only one study conducted on Q fever on water buffalo, in the neighbour provinces which Afyon has a border such as Konya, Burdur, Eskisehir were proven to be positive for the presence of Q fever among the tested cattle, sheep (Kilic *et al.* 2005, Gazyagci *et al.* 2011, Ozturk *et al.* 2012, Gunaydin *et al.* 2015). In addition to resistance of *C. burnetii* spores to environmental conditions, Afyon localized on a risky geographical location and the continental climatic features of Afyon, contributes the dispersion of aerosols in the province (Nakuene *et al.* 2004).

Eight (8.69%) positive ELISA titers and negative PCR results showed us that the antibody positive water buffaloes were in the chronic stage of the infection. The fact that the positive sera were not tested by FAT, we could not discriminate whether the antibodies developed against phase I or phase II. Persisting high level antibodies were attributed to continuous antigenic stimulation and considered to be indicative for chronic Q fever in humans (Wegdam-Blans *et al.* 2012). However in animals this situation is not well evaluated. From our point of view, the positive antibody titers cannot be attributed to onset of Q

fever infection because blood samples were negative for the presence of *C. burnetii* DNA. As previously declared by Musso and Raoult (1995) blood samples were suitable in the peracute stage of the infection in order to detect *C. burnetii* DNA. Nevertheless, the positive ELISA titers were lead us to think that those water buffaloes might have been infected with *C. burnetii* at any stage of their life as emphasized.

To sum up, the positive ELISA titers led us to think that climatic features of Afyon and Q fever prevalence of the neighbour provinces prone to water buffaloes to Q fever in Afyon. However, more extensive prevalence studies need to be carried out to define the role of buffaloes as reservoirs for this pathogen and also the role of *C. burnetii* as an abortive agent in this animal. Animal breeders should be informed to take hygienic measures in order to restrict the probable infection.

CONCLUSION

Seropositivity in water buffaloes had a ratio that should not be ignored. Q fever positivity in neighbour provinces which has a border to Afyon and both geographical and climatic characteristics of Afyon prone the water buffaloes to Q fever. Therefore, more detailed investigations should be carried out on water buffaloes.

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