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Detection of *Coxiella burnetii* in Poultry Egg samples in Iran Using Nested PCR Assay

[1]Ebrahim Rahimi and [2]Abbas Doosti
[1]Department of Food Hygiene, Faculty of Veterinary Medicine, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran
[2]Biotechnology Research Center, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran

Corresponding Author: Ebrahim Rahimi, Department of Food Hygiene, Faculty of Veterinary Medicine, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran

ABSTRACT

Q-fever is a widespread zoonosis caused by the obligate intracellular microorganism *Coxiella burnetii*. The epidemiology of Q-fever in Iran is essentially unknown. For this reason, this study for the presence of *C. burnetii* in hen, duck, goose, quail and ostrich sell egg samples in Isfahan, Gellan and Mazandaran provinces, Iran was conducted. In the present study, 369 hen, duck, goose, quail and ostrich sell egg samples from 15 villages of Isfahan, Gellan and Mazandaran provinces, Iran were tested for *C. burnetii* using a nested PCR assay. In total, 2 of 130 (1.5%) hen egg samples were positive; the positive samples originated from 1 of 15 (6.6%) villages. Eight of 104 (7.7%) duck egg samples from 2 villages were positive for *C. burnetii*. As for 34 gooses, 70 quail and 31 ostrich egg samples; they were all tested negative for *C. burnetii*. To our knowledge, this study is the first report of direct identification of *C. burnetii* using PCR in hen and duck egg samples in Iran. Further intensive prevalence studies on *Coxiella* infection and on possible dangers of poultry products will be needed to elucidate the epidemiology of Q-fever in Iran.

Key words: *Coxiella burnetii*, PCR, egg, poultry, Q-fever, Iran

INTRODUCTION

Q-fever, a zoonosis caused by the obligate intracellular *Coxiella burnetii* is endemic throughout the world. The microorganism affects arthropods, birds and pets, domestic and wild mammals as well as humans (Maurin and Raoult, 1999; Parker *et al*., 2006). In human, infection is often asymptomatic and in its mild form can be mistaken for other flu-like illnesses. Acute Q-fever presents mainly as atypical pneumonia or hepatitis (Arricau-Bouvery and Rodolakis, 2005) and chronic infection, however, is a severe disease that requires prolonged antibiotic therapy, because the infection can result in endocarditic (Zhang *et al*., 1998). Q-fever is essentially an airborne disease. Infections occur after inhalation of aerosols generated from infected animal placentes, body fluids or contaminated dust resulting from contaminated manure and desiccation of infected body fluids (Arricau-Bouvery and Rodolakis, 2005; Fretz *et al*., 2007). Since, birds are part of the host spectrum of *C. burnetii*, infected domestic poultry can transmit the agent to humans by contaminated raw eggs or through formites (Hirai and To, 1998).

Various methods have been used to identify *C. burnetii* infected animals and food samples including microscopic examination, isolation in laboratory animals, immunodiagnostic tests and Polymerase Chain Reaction (PCR) assays. Microscopic examination has poor diagnostic specificity

and sensitivity (Arricau-Bouvery and Rodolakis, 2005; Kazar, 2005) and bacteriological isolation is time-consuming and requires confined level-3 laboratories. Recently, PCR assay has become a very useful method for the detection of *C. burnetii* DNA in biological samples (Uwatoko *et al.*, 1996; Lorenz *et al.*, 1998; Berri *et al.*, 2003; Ongor *et al.*, 2004; Guatteo *et al.*, 2006).

The epidemiology of Q-fever in Iran is essentially unknown and to the authors' knowledge, the prevalence rate of *C. burnetii* in poultry eggs in Iran has never been reported. The objective of the present study was to determine the prevalence rate of *C. burnetii* in chicken, duck, goose, quail and ostrich sell egg samples in Isfahan, Gellan and Mazandaran provinces, Iran using a nested PCR assay.

MATERIALS AND METHODS

Collection of samples: Overall, 369 chicken (n = 110), duck (n = 104), goose (n = 34), quail (n = 70) and ostrich (n = 31) sell egg samples from 15 villages of Isfahan, Gellan and Mazandaran provinces, Iran from September 2009 to September 2010 were randomly selected. The samples were immediately transported to the laboratory in a cooler with ice packs and were processed within an hour of collection.

PCR detection of *C. burnetii*: *C. burnetii* was isolated from sell egg samples as described previously by Fretz *et al.* (2007). Purification of DNA was achieved using a genomic DNA purification kit (Fermentas, GmbH, Germany) according to the manufacturer's instruction and the total DNA was measured at 260 nm optical density according to the method described by Sambrook and Russell (2001).

All oligonucleotide primers were obtained from a commercial source (Cinna Gen, Iran). The nested PCR assay used to screen for *C. burnetii* was designed from the nucleotide sequence of the *com1* gene encoding a 27-KD outer membrane protein (OMP) as previously described (Zhang *et al.*, 1998) and the amplification was carried according to the method described elsewhere (Fretz *et al.*, 2007). For the nested PCR assay with primers OMP1-OMP2 and OMP3-OMP4, the first amplification was performed in a total volume of 25 μL containing 5 μL of DNA sample, 0.5 mM $MgCl_2$, 0.2 mM (each) dNTPs, 1 μM primer OMP1, 1 μM primer OMP2 and 0.5 U/reaction of Taq DNA polymerase (Roche Applied Science, Germany).

The PCR assay was performed at 94°C for 4 min and then for 30 cycles of 94°C for 1 min, 56°C for 1 min and 72°C for 1 min in a DNA thermal cycler (Master Cycler Gradiant, Eppendrof, Germany). In the second amplification, the reaction was performed in a total volume of 25 μL containing 2 μL of DNA sample, 0.5 mM $MgCl_2$, 0.2 mM (each) dNTPs, 0.8 μM primer OMP3, 0.8 μM primer OMP4 and 0.5 U/reaction of Taq DNA polymerase. The PCR assay was performed at 95°C for 4 min and then for 30 cycles of 94°C for 1 min, 57°C for 1 min and 72°C for 1 min. The PCR-amplified products (OMP1-OMP2: 501 bp; OMP3-OMP4: 438 bp) were examined by electrophoresis in a 1.5% agarose gel, stained with a 1% solution of ethidium bromide and examined under UV illumination. In the present study, *C. burnetii* DNA (Serial Number: 3154; Genekam Biotechnology AG, Germany) and DNase free water were used as the positive and negative controls, respectively.

Statistical analysis: Data were transferred to a microsoft excel spreadsheet (Microsoft Corp., Redmond, WA, USA). Using SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA), a Pearson chi-square test and Fisher's exact two-tailed test analysis was performed and differences were considered significant at values of $p<0.05$.

Table 1: Prevalence of *Coxiella burnetii* in chicken, duck, goose, quail and ostrich egg samples in Isfahan, Gellan and Mazandaran provinces, Iran

	No. of villages in the study regions	No. of samples per village	No. of egg samples	No. (%) of *C. burnetii* positive samples
Chicken	15	1-4	130	2 (1.5)
Duck	14	2-4	104	8 (7.7)
Goose	7	1-3	34	0 (0.0)
Quail	6	2-5	70	0 (0.0)
Ostrich	4	1-3	31	0 (0.0)

RESULTS AND DISCUSSION

In the present study, 369 chicken, duck, goose, quail and ostrich egg samples from 15 villages of Isfahan, Gellan and Mazandaran provinces, Iran were tested for *C. burnetii* using a nested PCR assay. In total, 2 of 130 (1.5%) hen egg samples were positive; the positive samples originated from 1 of 15 (6.6%) villages. Eight of 104 (7.7%) duck egg samples from 2 villages were positive for *C. burnetii*. As for 34 gooses, 70 quail and 31 ostrich egg samples; they were all tested negative for *C. burnetii* (Table 1). No significant differences in the prevalence rates ($p>0.05$) were observed between egg samples isolated in Isfahan, Gellan and Mazandaran provinces.

The prevalence of *C. burnetii* in hen and duck egg samples observed in this study (1.5% and 7.7%, respectively) is similar to a recent report in Japan that showed a prevalence of *C. burnetii* of 4.2% in shell eggs and 17.6% in mayonnaise samples using a PCR assay (Tatsumi *et al*., 2006). In a study conducted in the Switzerland, all 504 sell egg samples were negative for *C. burnetii* using a nested PCR assay (Fretz *et al*., 2007). In another study conducted in Japan, all of 200 chicken egg samples collected from supermarkets in Tokyo were negative by the nested PCR method (Hirai *et al*., 2005). The discrepancy between the different studies might be explained by the fact that the analyzed eggs were produced in different regions.

The findings of the present study are limited to PCR-based detection of *C. burnetii* DNA in poultry egg samples, so we are unable to speculate on the viability of organisms in egg samples, or on the sensitivity and specificity of the nested PCR assay compared to other diagnostic methods. The results of this investigation suggest that Q-fever should be considered as a possible case of unexplained fever in Iran, particularly among veterinary peoples and farmers.

CONCLUSION

Although no extensive prevalence study was undertaken, our results indicate that *Coxiella burnetii* infection appears to be relatively frequent in domestic duck eggs in Iran. Therefore, the results of this study indicate that domestic duck eggs are important sources of *C. burnetii* infection in Iran. To our knowledge, this study is the first report of direct identification of *C. burnetii* using PCR in chicken, duck, goose, quail and ostrich sell egg samples and the first report of direct identification of *C. burnetii* in chicken and duck sell egg samples in Iran. Further intensive prevalence studies on *Coxiella* infection in poultry foodstuffs will be needed to elucidate the epidemiology of Q-fever in Iran.

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