

Mini Review: Q Fever (Coxiellosis): Epidemiology, Pathogenesis and Current Laboratory Diagnosis

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Abstract

Q fever is zoonosis with global distribution caused by the strictly intracellular bacterium *Coxiella burnetii*. Causative agent of Q fever is an obligate intracellular parasite, classified in the genus *Coxiella*, family *Coxiellaceae*, class *Gammaproteobacteria*. The importance of the disease was assessed both in terms of human health and the serious economic damage they cause on livestock. Clinical manifestation of Q fever in humans is characterized by a wide variety - from asymptomatic infection to a chronic disease that can be fatal. Several basic methods have been developed to detection of *C. burnetii*. PCR and *C. burnetii* genomic sequences in whole blood are a sensitive and safe method of detection, with >90% sensitivity. A four-fold or greater rise of (CF) antibody (phase 2) between the paired sera is also diagnostic approach. Sensitivity of a four-fold rise in titre has been estimated as 73% ÷78% and specificity has been estimated as 90%, respectively. EIA is method with highly sensitive and specific. EIA detect IgM and then IgG antibodies which develop to phase II antigens in 10 to 14 days from symptom onset. IFA tests are of particular value for confirmation of acute infection and for diagnosis of chronic infection with high sensitivity. The technique detected IgG, IgM and IgA immunoglobulin classes. Suitable specimens for C.

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burnetii detection are blood samples. Although scientific interest in Q fever has always existed, a number of facts concerning the unforeseen nature of the epidemic, various clinical manifestations both in humans and in animals, the opportunities for chronic and other features of infection remain unclear. For this reason, timely and highly sensitive laboratory diagnosis is crucial for the outcome of the disease and subsequent treatment and monitoring.

Keywords: *Coxiella burnetii*; Q fever; PCR; EIA IgM/IgG; IFA.

1. Introduction

Zooantroponozes are constantly growing group of infections including diseases manifesting in the form of epizootic outbreaks and epidemics, with serious human health, environmental, economic and socio-domestic consequences. Rapidly advancing climate changes brought about by global warming, more intense trade in live animals and globalizing world intensified existing natural and antropurgic outbreaks of several diseases with zoonotic potential and created conditions for expanding the area of the spread of other infections having status for exotic infections in countries in the temperate zone of the planet. These diseases are among the priority areas of the World Health Organization (WHO), the European Center for Disease Prevention and Disease Control (ECDC) and the European Agency for Food Safety (EFSA).

2. Epidemiology and molecular genetic

Q fever is zooantroponozis with global distribution caused by the strictly intracellular bacterium *Coxiella burnetii*, established on all continents, with the exception of New Zealand [1]. The host range susceptible to infection types is too wide and includes a large number of wild and domesticated mammals and many birds and amphibians, and the ability of ticks stored and transmitted as vectors infectious agent associated with the formation and maintenance for an extended period during natural outbreaks [2; 3; 4]. *Coxiella burnetii* is included in category "B" list of potential biological weapons [5]. Causative agent of Q fever is an obligate intracellular parasite, classified in the genus *Coxiella*, family *Coxiellaceae*, class *Gammaproteobacteria*. There is a phase variation in the development of *C. burnetii* which allows the serological differentiation between an acute and a chronic form. The main structural elements of *C. burnetii* are: cytoplasm, nucleic and surface structures [6]. Its genome contains one molecule of DNA with sizes from 1.5 to 2.4 x 10⁶ base pairs, the length of which varies in different strains as well as one of the five types of plasmids (QpHI, QpRS, QpDG, QpDV and one anonymous) (30-51 kbp), which constitute about 2% of the total genetic information [7]. For the rapid detection and identification of *C. burnetii* have been developed and widely used a complex of methods: serological (immunofluorescence, CFT, ELISA etc.), cell culture, and molecular biological (conventional, nested, real-time PCR, etc.) [8]. The introduction of PCR significantly increased the sensitivity and specificity of early etiological diagnosis and sequencing of genomic regions from different strains of *C. burnetii*, permit their differentiation and characterization with regard to such important biological properties, responsible for the virulence of the agent as adhesion, penetration, intracellular transport, "escape" from the immune response, pathogenesis and others [9; 10]. There are 2134 identified gene sequences of the genome of *C. burnetii* so far, 719 (33.7%) of them are unique [7]. In the global gene bank there are more than 30 genotypes *C. burnetii*, allocated on the basis

of phylogenetic analysis at 6 genogroups (I - VI), some of which occur everywhere in endemic and non-endemic areas [11; 12]. Different genotypes vary by pathogenic properties, clinical outcome and noso-geographical spread, but the data are conflicting [13]. Regardless of the early reports of applicability of various methods (MLVA, MST, RFLP, etc.) for genetic identification and detection of specific gene determinants of *C. burnetii*, encoding the factors of virulence/pathogenicity, resistance to antibiotics, etc. [14; 15], their information is incomplete and does not provide a definite answer. The importance of the disease was assessed both in terms of human health and the serious economic damage they cause on livestock. For the first time the disease was described in humans by E. Derrick in 1935, which gave the name "Q fever" (from eng. "query" - "unclear") [16], followed by numerous other reports. The infection is endemic in almost all countries in Europe, where they are registered epidemic outbreaks with different sizes, as well as varying the degree of infestation of farm animals [13; 17].

3. Pathogenesis, transmission and tropism

Clinical manifestation of Q fever in humans is characterized by a wide variety - from asymptomatic infection to a chronic disease that can be fatal. In 40% of clinically manifested cases of acute Q-infection was observed self-limiting febrile illness [18] and/or primary atypical pneumonia [4], it is possible in some cases to develop granulomatous hepatitis as well [19]. Of particular note is the persistent form of *C. burnetii* infection, which is severe, with high lethality [20]. Q rickettsial endocarditis is the most common form of chronic infection, patients with valvular and vascular lesions are at high-risk [21]. Many people infected with Q fever never show symptoms. If you do have symptoms, you'll probably notice them between three and 30 days after exposure to the bacteria. Signs and symptoms may include:

- High fever, up to 105 F (41⁰C)
- Severe headache
- Fatigue
- Chills
- Cough
- Nausea
- Vomiting
- Diarrhea
- Sensitivity to light

In animals, the disease usually is subclinical. In ruminants, however, infection can occur with miscarriages, birth of non-viable offspring and reproductive problems, leading in some cases to serious economic losses. Some data suggest that Coxiellosis in animal, possibly in association with other factors of an infectious nature may manifest with other clinical characteristics (pneumonia, mastitis), but validation of any opinion requires further research, including experimental. Excretion of the agent can last more than four months. This fact is crucial, since the infected animals are the main source of infection in people. The main role in this play productive ruminants (cows, sheep and goats) that emit huge amount of *C. burnetii* in amniotic fluid and placentas at normal birth or in cases of abortion. Although to a lesser extent, the causative agent is excreted in milk, urine and

faeces. Other domestic animals and birds have less participation in epidemiological process. The mechanism of transmission of *C. burnetii* in humans is realized mainly by inhalation of aerosol particulates, contaminated with the pathogen [22] and, more rarely alimentary. The bite of infected ticks has its epidemiological significance to natural outbreaks.

4. Laboratory Diagnosis

Several basic methods have been developed to detection of *C. burnetii* [23-26] (Fig 1).

4.1 Direct detection of *C. burnetii*

Stains and direct immunofluorescence for light microscopy and electron microscopy are not performed routinely. *C. burnetii* are highly infectious and unfixed tissue samples for examination for *C. burnetii* should be handled in a biohazard cabinet until fixed with formalin, dehydrated methanol or acetone. *C. burnetii* cultures are risk level 3 organisms and should only be handled by immune staff in a PC3 facility [28].

4.2 Polymerase chain amplification (PCR)

PCR of *C. burnetii* genomic sequences in whole blood a sensitive and safe method of detection [27]. The initial extraction of the DNA involves procedures – proteinase K treatment, chloroform phenol extraction, heating over 90°C – which inactivate viable *C. burnetii*. *Test Sensitivity and Specificity* - This method has >90% sensitivity compared with serodiagnosis early in infection. It is highly specific. Sensitivity decreases with progression of the illness with no detection after day 17. However, sensitivity in chronic Q fever is only 50-60%. *Suitable Specimens* -Tissue, whole blood, serum. *Suitable Test acceptance/validation criteria* - In run and positive and negative control (non-template controls), extraction control.

4.3 Serological diagnosis

Historically, the most frequently used technique was complement fixation with *C. burnetii* Phase 2 antigen, and later, in addition, with Phase 1 antigen. Phase 2 antibody is the first to be detected and phase 1 antibody is invariably present in chronic Q fever infection, including endocarditis, with or without phase 2 antibody. Examination of acute and convalescent sera fulfils the most reliable criteria for an acute infection: a four-fold or greater rise of (CF) antibody (phase 2) between the paired sera is diagnostic. The technique has the limitations that it is labour intensive and does not measure IgM so that a positive result may not be obtained until late in the disease. Also, it does not measure IgA antibody which may be important in Q fever endocarditis [29; 30]. *Test Sensitivity and specificity* - CFT measures mainly IgG so may not be positive early in acute Q fever when the patient first presents and only IgM antibody may be present. The test should be repeated in 2-4 weeks and run in parallel with the first sample to determine titre changes. Sensitivity of a four-fold rise in titre has been estimated as 73% ÷78% and specificity has been estimated as 90%, respectively. *Suitable specimens* - Blood samples. Ideally acute and convalescent samples should be provided, especially in acute Q fever, to monitor titre changes.

Suitable test acceptance/validation criteria - Use of internal and external controls and test performance should

comply with method.

4.4 Enzyme immunoassay (EIA) Testing

Commercial kits are available to measure IgM and IgG antibodies to phase 2 antigens. If only a single convalescent serum is available it may be difficult to distinguish persistent IgM from a past infection from that of a current infection, as not all Q fever cases make the IgM to IgG switch. IgM has been observed to persist for over 600 days after an acute Q fever infection. While acute primary Q fever is confirmed with reasonable efficiency with the EIA test it is unwise to rely solely on this method if chronic Q fever infection is suspected and where interpretation has to be based on the height of titers to Phase 1 and 2 antigens in the IgG and IgA classes. These tests are not quantitative so should be used only as a screening test to select samples for the more labour intensive CFT or IFA testing. IgM and then IgG antibodies develop to phase II antigens in 10 to 14 days from symptom onset. Seroconversion or a fourfold rise in phase II IgG or CFT titre in convalescent serum is diagnostic of Q fever. *Suitable specimens* – Blood samples. Ideally acute and convalescent samples should be collected and tested in parallel. *Test sensitivity and specificity* - Published data suggest that EIA is highly sensitive and specific. Data regarding commercial assays should be sought from the manufacturer. *Suitable test acceptance/validation criteria* - Follow manufacturer’s instructions.

4.5 IFA testing

IFA tests are of particular value for confirmation of acute infection and for diagnosis of chronic infection. The technique involves titrating sera by immunofluorescence (IFA) on microdots of *C. burnetii* expressing phase 1 or phase 2 antigens with conjugates directed against IgG, IgM and IgA immunoglobulin classes [31]. *Suitable Specimens* - Blood samples. Sequential samples should be tested in parallel. *Test Sensitivity and Specificity* - The microdot phase 1 and 2 IgM, and IgG and the IgA tests are highly sensitive and specific for the detection of Q fever antibodies.

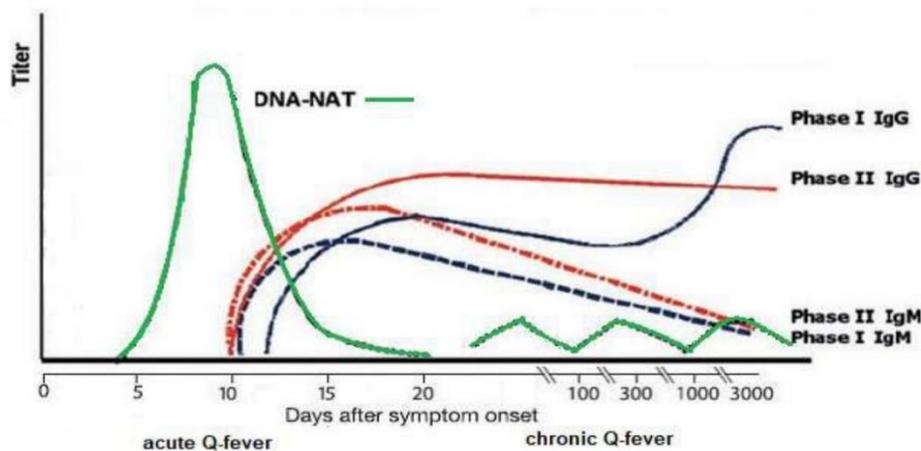


Figure 1: Typical serological and molecular response in acute and chronic Q fever

5. Conclusion

Although scientific interest in Q fever has always existed, a number of facts concerning the unforeseen nature of the epidemic, various clinical manifestations both in humans and in animals, the opportunities for chronic and other features of infection remain unclear. Q fever prevalence is unknown and it is likely that the number of cases of is underestimated. At the same time changes in migration processes, the threat of bioterrorism, the problems in national and global scale associated with environmental and other factors increased the risk of spreading morbidity in emerging noso-geographical areas. Therefore, the diagnosis must be considered in the case of an unexplained fever, especially if the fever recurred following contact with possibly contaminated mammals. The best tests for diagnosis are those which permit the direct detection of bacteria. They include shell vial culture, PCR amplification, and immunodetection with tissue biopsy specimens. All these techniques require a level 3 biosafety laboratory and trained personnel due to the extreme infectivity of *C. burnetii*. In chronic cases, the techniques that allow the direct detection of *C. burnetii* in blood or tissues should be used before the beginning of therapy. As for indirect specific diagnosis, the technique to be used should be very sensitive and should detect antibodies early in the course of the disease. Although many techniques have been described, immunofluorescence assay is the reference method. It is both very specific and sensitive. The presence of cross-reacting antibodies should be investigated by cross-adsorption followed by Immunoblot testing [32]. We recommend that all patients with possible *C. burnetii* infection to be tested by combination of serological and molecular assays and be monitored for their immune status and persistent infection.

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Reference

- [1]. Parker, N., J. Barralet, A. Bell. (2006). "Q fever". *Lancet*, 367, 679 – 688.
- [2]. Porter S, Czaplicki G, Mainil J, Guatteo R, Saegerman C. (2011). 'Q Fever: Current State of Knowledge and Perspectives of Research of a Neglected Zoonosis'. *Int J Microb*, Art. ID 248418, 22 pages
- [3]. Ruiz-Fons F, Rodriguez O, Torina A, Naranjo V, Gortazar C, Fuente J. (2008). "Prevalence of *Coxiella burnetii* infection in wild and farmed ungulates". *Vet Microb*, 126, 282 – 286.
- [4]. Maurin, M. & D. Raoult. (1999). "Q Fever". *Clin Microb Rev*, 12, 518 - 533.
- [5]. Kagawa F., Wehner J, Mohindra V. (2003). "Q fever as a biological weapon". *Eur PMC*, 18, 183 - 195.
- [6]. Fournier P., Marrie T, Raoult D (1998). "Diagnosis of Q fever". *J Clin Microb*, 36, 1823 – 1834.
- [7]. Angelakis E. & Raoult D. (2010). "Q fever". *Vet Microb*, 140, 297 - 309.
- [8]. OIE Terrestrial Manual, 2.1.1.6. Chapter Q fever. (2015)
- [9]. Sekoyova Z, Roux V, Raoult D. (1999). "Intraspecies diversity of *Coxiella burnetii* as revealed

- by com1 and mucZ sequence comparison". FEMS Microbiol Lett, 180, 61- 67.
- [10]. Seshadri R, Paulsen I, Eisen J, Read T, Nelson K, Nelson W, Ward N. et al. (2003). PNAS, 100, 5455 – 5460.
- [11]. Glazunova O, Roux V, Freylikman O, et al. (2005) "Coxiella burnetii genotyping". Emerg Inf Dis J, 11, 1211– 1217.
- [12]. Jager C., Willems D, Thiele G. Baljer (2002). "Molecular characterization of Coxiella burnetii isolates". Epid & Inf, 120, 157 – 164.
- [13]. Tilburg J, Rossen J, Hannen E. van, et al. (2012). " Genotypic diversity of Coxiella burnetii in the 2007–2010. Q fever outbreak episodes in the Netherlands". J Clin Microbiol, 50, 1076 – 1078.
- [14]. Hendrix L, Samuel J, Mallavia L. (1991). "Differentiation of C. burnetii isolates by analysis of restriction-endonuclease-digested DNA separated by SDS-PAGE". J Gen Microb, 137, 269–276.
- [15]. Valkova, D. & J. Kazar. (1995). "A new plasmid (QpDV) common to Coxiella burnetii isolates associated with acute and chronic Q fever". FEMS Microbiol Lett, 125, 275 – 280.
- [16]. Derrick E. (1937). "The course of infection with Coxiella burnetii". Med J Austr, 1, 1051 - 1057.
- [17]. EFSA Panel on Animal Health and Welfare, EFSA Panel on Biological Hazards. Scientific Opinion on Q fever. (2010). EFSA Journal, 8, 1595 – 1667.
- [18]. Tissot-Dupont H, Raoult D, Brouqui P, et al. (1992). "Epidemiologic features and clinical presentation of acute Q-fever in hospitalized patients – 323 French cases". Am J Med, 93, 427 - 434.
- [19]. Tjwa M, De Hertogh G, Neuville B, et al. (2001). "Hepatic fibrin-ring granulomas in granulomatous hepatitis: report of four cases and review of the literature". Acta Clin Belg, 56, 341 – 348.
- [20]. Honarmand, H. (2012). "Q fever: an old but still a poorly understood disease". Inter Persp Inf Dis, Article ID 131932, 8 pages.
- [21]. Morguet A, Jansen A, Raoult D, Schneider T. (2007). "Late relapse of Q fever endocarditis". Clin Res Cardiol, 96, 519 – 524.
- [22]. Tissot-Dupont H, Amadei M, Nezri M, Raoult D. (2004). "Wind in November. Q fever in December". Emerg Infect Dis, 10, 1264 – 1269.
- [23]. Scola B. L. (2002). "Current laboratory diagnosis of Q fever. Seminars in pediatric infectious diseases". 13(4), 257–262. Available: <https://doi.org/10.1053/spid.2002.127199>
- [24]. Bartelink A, Stevens H, van Kregten, E, Meijer J, Beeres M, & van Deuren M. (2000). "Acute en chronische Q-koorts; epidemiologie, symptomen, diagnostiek en therapie van infecties met Coxiella burnetii [Acute and chronic Q fever; epidemiology, symptoms, diagnosis and therapy of infection caused by Coxiella burnetii]". Nederlands tijdschrift voor geneeskunde, 144(27), 1303–1306.
- [25]. <https://www.cdc.gov/mmwr/preview/mmwrhtml/rr6203a1.htm>
- [26]. Graves S, Massung R. (2015). "Coxiella. In: Manual of Clinical Microbiology". 11th Edition ASM Press.
- [27]. Schneeberger P, Hermans M, van Hannen E, Schellekens J, Leenders A, Wever P. (2009, Dec). "Real-time PCR with serum samples is indispensable for early diagnosis of acute Q fever". Clin Vaccine Immunol. 17(2):286-90. Available: <https://doi: 10.1128/CVI.00454-09>.
- [28]. Musso D, Raoult D. (1995). "Coxiella burnetii blood cultures from acute and chronic Q-fever patients". J. Clin. Microbiol. 33:3129 –3132.

- [29]. Dupuis G, Péter O, Peacock M, Burgdorfer W, Haller E. (1985, Oct). "Immunoglobulin responses in acute Q fever". *J Clin Microbiol.* 22(4):484-7
- [30]. Field P, Mitchell J, Santiago A, Dickeson D, Chan S, Ho D, Murphy A, Cuzzubbo A, Devine P. (2000, Apr). "Comparison of a commercial enzyme-linked immunosorbent assay with immunofluorescence and complement fixation tests for detection of *Coxiella burnetii* (Q fever) immunoglobulin". *M. J Clin Microbiol.* 38(4):1645-7.
- [31]. D'Harcourt S, Soto A, Burgos V, Calero D, Martínez-Zapico R. (1996, Sep). "Comparison of immunofluorescence with enzyme immunoassay for detection of Q fever". *Eur J Clin Microbiol Infect Dis.* 15(9):749-52.
- [32]. Fournier, P. E., Marrie, T. J., & Raoult, D. (1998). Diagnosis of Q fever. *Journal of clinical microbiology*, 36(7), 1823–1834. <https://doi.org/10.1128/JCM.36.7.1823-1834.1998>