

SERION ELISA *classic*
Coxiella burnetii Phase 1 IgG/IgA
Coxiella burnetii Phase 2 IgM/IgG

1. INTRODUCTION
2. PATHOGEN
3. EPIDEMIOLOGY
4. CLINICAL MANIFESTATIONS
5. PROPHYLAXIS AND THERAPY
6. DIAGNOSIS
 - 6.1 Immune response
 - 6.2 Serodiagnosis
 - 6.3 Fields of Application
7. BIBLIOGRAPHY

1. INTRODUCTION

By definition the term "zoonosis" summarizes infectious diseases which are caused by the direct contact with animals and which are accidentally transmitted by animals - mostly by arthropodes - to the human host. Especially persons are at risk to acquire zoonosis who are particularly exposed to the animal vectors during their professional activities. Besides lyme borreliosis and leptospirosis, these features are also true for infection with *Coxiella burnetii* which had been first described in 1937.

2. PATHOGEN

The causative infectious microorganism - *Coxiella burnetii* - belongs to the family of the RICKETTSIACEAE. The gram-negative bacteria are pleomorphic and adapted evolutionary to an obligate intracellular life within host cells. Generally rickettsia cannot be cultivated in vitro in synthetic media.

Due to its inherent resistibility to environmental stress conditions the bacteria remains infectious for a long period of time. After being phagocytosed by macrophages the bacteria multiply within phagolysosomes, which normally are formed to kill infectious agents. This mechanism to evade the protective mechanism of the host explains partly the enormous resistibility of the microorganisms to environmental stress.

3. EPIDEMIOLOGY

Q-fever diseases are spread world-wide. In central Europe Q-fever appear sporadic or as small limited epidemic episodes.

Coxiella burnetii was detected in various mammals, birds and arthropodes. In contrast to other members of the rickettsia group *Coxiella burnetii* can be transmitted independent of ticks, which normally are the main hosts.

The coxiella are highly contagious.

Approximately 90% of all infections are caused by the inhalation of contaminated dust containing dried animals excretions. Further sources for Q-fever disease are contaminated native milk, skin contact with infected animal organs, contaminated clothes as well as infected ticks (*Dermacentor marginatum*). The transmission from human to human was found only rarely.

Up to 2,5% of life stock and pets like dogs and cats are infected with *Coxiella burnetii*. Professions, which are featured by a high exposition risk to the pathogen are listed below.

- Personnel in agriculture
- Personnel in life stock production
- Personnel of slaughter-houses
- Personnel in milk production
- Personnel in animal skin processing industry
- Veterinary surgeons
- Laboratory personnel

In person groups with professions as listed above seroprevalence can rise up to 65% (farmers, veterinary surgeons). In contrast in normal population seroprevalence is about 20% only.

Q-fever diseases and subsequent lethal outcomes in Germany have to be reported to the National Department of Health .

On the basis of the official reports in Germany incidence of Q-fever diseases is 27 - 100 cases per year. However, due to the mostly mild courses of the disease and the false diagnosis of "Flu-like disease", acute as well as chronic infections seem to be diagnosed less. In the majority of cases serodiagnosis is not applied.

4. CLINICAL MANIFESTATIONS

After an incubation period of up to 30 days the disease shows acute clinical symptoms like the sudden onset of fevers with high temperatures above 40 °C, severe headache and myalgia. Additionally symptoms of interstitial pneumonia mostly occur. In general no exanthema are observed. In the majority of cases the Q-fever

disease is limited to an acute infection. Clinical complications, which could affect nearly any organ, are seen rarely. Between 40% and 70% of the infection courses are clinically inapparent or with only sub-clinical manifestations.

Chronic courses of Q-fever occur only rarely. In the majority of the cases life-threatening endocarditis and granulomatous hepatitis are observed. The clinical manifestations often appear years after disappearance of the acute symptoms.

5. PROPHYLAXIS AND THERAPY

Within person groups who are at high risk due to their profession effective protection mechanisms should be applied in order to prevent a direct and immediate contact with contaminated objects. (gloves, caution versus native animal products). Furthermore immune prophylaxes predominantly for veterinary surgeons and laboratory personnel may be applied; however general use is not recommended.

Both, acute and chronic infections can be treated with tetracyclines and chloramphenicol. For maximal recovery chronic courses of the diseases have to be treated for years.

6. DIAGNOSIS

The clinical symptoms (i.e. interstitial pneumonia) and chronic Q-fever disease are unspecific. Therefore a wide spectrum of other diseases should be regarded in *differential diagnosis*:

- Flu-like diseases
- leptospirosis
- meningitis of various origin
- atypical (interstitial) pneumonia of viral and bacterial origin

Due to the high personnel and time effort for detection of *Coxiella burnetii* by cultivation and also due to the high pathogenic potential of this particular microorganism culture techniques are

of no practical importance for laboratory diagnosis.

Antigen in swabs and biopsy specimens can be detected with Fluorescein-labelled specific monoclonal and polyclonal antibodies.

6.1 Immune response to *Coxiella burnetii*

Like typical gram-negative bacteria *Coxiella burnetii* expose lipopolysaccharide (LPS) structures on the cell surface. In dependence on environmental conditions an antigenic phase variation is detected. During natural infection in vivo and also under low-passage conditions in vitro (μ 10 passages) exclusively hydrophilic phase 1-LPS molecules are detectable on the cell surface. In contrast, under high-passage conditions phase 2-LPS can be detected.

It is known that the phase 1-LPS structures under in vitro conditions prevent the cell lysis within the phagolysosomes of the macrophages.

During natural infections the phase 2 molecules seem to be highly immunogenic as compared with the phase 1 structures; additionally the phase 1-LPS molecules are masked in acute infection and therefore not exposed to the host's immune system. As a consequence only anti-phase 2 IgM and IgG antibodies can be detected in the early acute disease. In the time course of the development of chronic disease anti-phase 1 IgG and IgA antibodies appear.

Due to technical problems in the in vitro large scale production of phase 1 antigens, commercially available serodiagnostic test systems were exclusively based on phase 2 antigens.

As phase 2-specific antibodies persist through the chronic events the exclusive use of phase 2 antigen in serodiagnosis do not enable the discrimination between acute and chronic Q-fever disease.

6.2 Serodiagnosis

In laboratory diagnosis of Q-fever various assay systems for the detection of *Coxiella burnetii* - specific antibodies are commonly used.

The complement fixation test (CFT) is the most widespread test system in routine diagnosis. As described above until recently no phase 1 antigens were commercially available. Therefore CFT was performed only on the basis of phase 2 antigens.

In addition to phase 2 antigens Serion Immundiagnostica GmbH offers phase 1 antigen for CFT. Applying this novelty a significant gap in the laboratory diagnosis of Q-fever infections is closed. Both antigens are applied to the well-known standardization of the CFT system of SERION Immundiagnostica GmbH. (see current price list)

Within a few weeks after onset of symptoms the phase 2 CFT becomes positive. During chronic Q-fever disease phase 1 CFT show increasing high antibody titers.

Due to the high automation, the inherent high sensitivity and the possibility of a differentiated insight into the antibody response ELISA techniques (Enzyme-linked immunosorbent assay) are of increasing importance.

With the SERION ELISA *classic* Coxiella burnetii phase 2 (IgM, IgG) and phase 1 (IgG, IgA), test systems for the differentiation of acute and chronic Q-fever diseases are commercially available for the first time.

In the following table characteristic serological features of the various stages and clinical manifestations of Q-fever disease are listed.

Immune Response

	Phase 2		Phase 1	
	IgG	IgM	IgG	IgA
Acute Infections	++	+ / ++	(+)	-
Chron. Infections:				
gran. Hepatitis	+++	++ / +++	+ / ++	- / +
Endocarditis	++ / +++	+ / ++	++ / +++	++ / +++

Legend: - no
+ low
++ moderate
+++ high

In contrast, strong IgA titer increases and high IgA titers directed against phase 1 antigens are relevant diagnostic features of endocarditis caused by chronic Q-fever infection. High IgG

antibody titers against phase 1 and phase 2 antigens accompany IgA response.

Acute Q-fever infections are featured by the appearance of phase 2-specific IgM antibodies. In general only low IgG titers against phase 1 and phase 2 antigens are detectable. Only rarely anti-phase I-IgM antibodies are seen.

6.3 Fields of application

- early detection of Coxiella burnetii-infections by phase 2-specific IgM* antibodies
- differential diagnosis of infections of the respiratory tract by phase 2-specific IgG and IgM* antibodies
- diagnosis of chronic Q-fever disease by phase 1-specific IgG and IgA antibodies as well as by phase 2-specific IgG and IgM* antibodies
- serological therapy follow-up in acute and chronic diseases by phase 1-specific IgG and IgA as well as phase 2-specific IgG and IgM* antibodies

* adsorption of Rheuma factor is recommended

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