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# СЛОЖНОСТИ ВЕРИФИКАЦИИ ДИАГНОЗА ЛИХОРАДКИ КУ ПРИ ОТРИЦАТЕЛЬНЫХ РЕЗУЛЬТАТАХ ПЦР-ТЕСТИРОВАНИЯ

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# Difficulties of Q Fever Diagnostic Verification at Negative PCR Testing Results

**Резюме**

**Цель работы:** продемонстрировать сложность верификации диагноза лихорадки Ку при отрицательных результатах ПЦР-тестирования на наличие в крови ДНК *Coxiella burnetii* и оценить встречаемость серологических маркеров среди пациентов, отобранных для настоящего исследования по совокупности клинико-эпидемиологических данных. **Материалы и методы:** у 111 пациентов методами иммуноферментного анализа и полимеразно-цепной реакции изучены образцы плазмы/сыворотки крови на наличие специфических антител и ДНК возбудителя. При выявлении антител к *C. burnetii* II фазы дополнительно проводились исследования на наличие IgG/IgA к коксиеллам I фазы, а также была изучена авидность специфических иммуноглобулинов класса G. **Результаты:** у 10 пациентов с отрицательными результатами полимеразно-цепной реакции были выявлены антитела к *C. burnetii*. В статье приведено подробное описание трех клинических случаев с лабораторным подтверждением инфицирования C. Burnetii на основании анализа полученных серологических профилей, титров специфических антител и оценки их авидности. **Заключение:** результаты исследования свидетельствуют о том, что отрицательные результаты ПЦР-тестирования не исключают у пациентов инфицирования *C. Burnetii*. В связи с этим, пациентам, у которых по клинико-эпидемиологическим данным не исключается лихорадка Ку, целесообразно назначение комплекса лабораторных исследований для верификации диагноза, предусматривающего не только исследования ДНК возбудителя, но и специфических антител. Для уточнения стадии заболевания и снижения риска развития осложнений коксиеллеза необходим мониторинг динамики титров антител к *C. burnetii* в I и II фазовых состояниях дифференциально. Оценка авидности антител будет полезна для понимания срока давности инфицирования *C. burnetii*.

***Ключевые слова:*** *лихорадка Ку, коксиеллез, антитела к Coxiella burnetiid*

**Конфликт интересов**

Авторы заявляют, что данная работа, её тема, предмет и содержание не затрагивают конкурирующих интересов

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**Abstract**

**Aim of the work**: to demonstrate the difficulty of verifying the diagnosis of Q fever with negative results of PCR (DNA of *Coxiella burnetii*) in the blood and to assess the occurrence of serological markers among patients selected for this study based on a combination of clinical and epidemiological data. **Materials and methods:** plasma/serum samples of 111 patients according to clinical and epidemiological data studied due ELISA and PCR for specific antibodies to *Coxiella burnetii* and DNA of pathogen. Additionally, in the presence IgG to *C. burnetii* phase II, IgG / IgA to phase I and the avidity of specific IgG were studied. **Results:** the specific antibodies to *C. burnetii* antigens at negative results of PCR detected in 10 cases. The article provides the description of three clinical cases for demonstration of difficulties of coxiellosis diagnosis with analysis of serological profiles, titers and avidity of antibodies. **Conclusion:** the results of the study indicate that negative results of PCR testing do not exclude *C. burnetii* infection. For patients who, according to clinical and epidemiological data, Q fever is not excluded, it is advisable to prescribe a complex of laboratory tests to verify the diagnosis, which includes not only studies of the pathogen's DNA, but also specific antibodies. To clarify the stage of the disease and reduce the risk of developing complications of coxiellosis, it is necessary to monitor the dynamics of antibody titers to *C. burnetii* in phase I and II phase states differentially.

***Key words:*** *Q fever, coxiellosis, antibodies to Coxiella burnetii*

**Conflict of interests**

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PCR — polymerase chain reaction

# Introduction

Q fever is a zoonosis, caused by the obligate intracellular bacterium *Coxiella burnetii*. It is widespread worldwide [1]. The disease was first described by Edward Holbrook Derrick in abattoir workers in Brisbane, Queensland (Australia) in 1933. He suggested calling this zoonosis "Q fever" ("Q" stands for "query") [2]. Specific features of Q fever include a variety of portals of entry, clinical polymorphism, subclinical course in a majority (up to 60 %) of patients and serious complications at the chronic stage [3]. The diversity of clinical manifestations in acute Q fever is associated with its mechanism of infection, infective dose and condition of the individual immune system [4].

The most common sources of infection are ruminant farm animals such as cattle, goats, and sheep. The mammals excrete Coxiella into the environment with feces, milk, and urine. The maximum amount of the causative pathogen is accumulated in the reproductive organs, resulting in premature births, abortions, and stillbirths in female animals [5]. Wild and domestic fowl can also be the source of infection, excreting the pathogen in feces. Ticks of different genera are reservoirs and carriers of the infectious agent in both natural and anthropogenic foci of Q fever [6–8]. High stability in the environment and resistance to various external factors allow for long-term persistence of the pathogen in the environment and spread of dusty aerosol with air currents over long distances. Humans can get infected with Q fever via fecal-oral, direct contact, and vector-borne routes of transmission [9, 10].

In Q fever, the incubation period varies 10 to 40 days, being 12 to 20 days on average. The disease onset is acute in 75 % of patients and is characterized by flu-like symptom complex. A polymorphic rash is observed in approx. 25 % of patients. Meningism events may occur at the height of fever. In acute Q fever, cardio-vascular involvement can be manifested as myocarditis, pericarditis, endocarditis, as well as heart rhythm disorders. Other possible signs of acute condition can be atypical pneumonia, hepatitis, pancreatitis, lymphadenopathy, extrapyramidal disorders, etc. Q fever progression to chronic form generally occurs in 3–6 months after acute infection and is reported in 5 % of patients on average. The chronic course is often complicated by Q fever endocarditis with heart valve involvement, aneurysms, vascular graft infection, vertebral osteomyelitis, hepatitis, etc. [11–16].

Based on molecular genetic, serological testing and the presence of clinical symptoms, some expert groups have attempted to describe diagnostic criteria for identification of acute and chronic stages of infection, assuming such terms as "proven," "probable," and "possible" in the latter case. A positive polymerase chain reaction (PCR) for pathogenic DNA in blood nearly always correlates with acute Q fever, however, the reaction quickly

becomes negative after initiation of antibiotics and build-up of specific antibodies. Therefore, PCR should be conducted within the first two weeks after clinical symptoms occur and until treatment with antibiotics is started. Nevertheless, this condition is rarely observed in practice [17, 18].

Serological testing is considered as the first-line diagnostic method. The immune response induces the production of antibodies to phase I and phase II *C. burnetii,* since the pathogen has antigenic variations associated with mutational change in lipopolysaccharide composition. The diagnosis of primary (acute) infection can be confirmed by a detected pronounced change over time in the levels of IgG and IgM phase II antibodies in paired sera taken at an optimal time interval. Antibodies to phase II *C. burnetii* are usually the first to be detected in the patient's blood and are most often detected 7–15 days after infection followed by a gradual decrease in levels, although remaining detectable for a long time [18]. The differential detection of immunoglobulins of different classes to *C. burnetii* antigens in phases I and II is of special importance. In some cases, the assessment of change in the levels of antibodies to phase I and II*C. burnetii* antigens in paired sera over time allows suggesting the stage of infection in a patient [19]. Titers of IgG to phase II antigens is generally greater than titers of IgG to phase I in current (acute) infection. In Q fever endocarditis and often in other manifestations of chronic condition, the titers (levels) of phase I *C. burnetii* IgG are almost always greater than the titers (levels) of phase II *C. burnetii* IgG [20]. Since there are still no clear diagnostic criteria for acute or chronic stages of the disease, some studies evaluating IgG avidity to *C. burnetii* in clinical practice have been recently published, suggesting that low-avid phase II *C. burnetiid* IgGs are in favor of recent infection. Higher levels of IgG avidity to phase I *C. burnetii* compared to IgG avidity to phase II *C. burnetii* are in favor of chronic Q fever [21, 22].

**The study was aimed** at demonstrating the difficulty of Q fever diagnosis verification in patients with negative PCR for *C. burnetii* DNA and assessing occurrence of serological markers in patients selected for the study by the aggregate of clinical and epidemiological data. The authors considered it necessary to provide a detailed description of three clinical cases of Q fever confirmed by serological methods, assessing profiles of antibodies to *C. burnetii.*

## Materials and Methods

Blood plasma/serum samples were collected from 111 patients, being examined and treated in the Infectious Diseases Hospitals in Moscow from April till October 2021, and studied for clinical and epidemiological data (presence of fever, fact of tick biting/crawling, etc.).

If a patient had fever, rash, and the tick removed from the patient showed markers of transmissible pathogens, the blood plasma was tested by real-time PCR to detect pathogens of borreliosis, tick-borne encephalitis, tick-borne rickettsiosis, human granulocytic anaplasmosis and monocytic ehrlichiosis using the kits produced by Central Research Institute for Epidemiology of the Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing: AmpliSens® TBEV, *B. burgdorferi sl, A. phagocytophilum, E. chaffeensis/E.muris*-FL, AmpliSens® *Coxiella burnetii*-FL, AmpliSens® *Rickettsia* spp. SFG-FL.

The patients' blood serum samples were also tested using reagent kits authorized in the Russian Federation for the following purposes:

- for screening of IgG/IgM to Q fever pathogen (kits: *Coxiella burnetii* ELISA IgG, *Coxiella burnetii* ELISA IgM, manufactured by Vircell S.L, Spain);
- for confirmation and differential identification of different classes of antibodies to phase I and II *Coxiella burnetii* using reagent kits, manufactured by Virion/Serion Institute, Germany: Virion/Serion *Coxiella burnetii* Phase I IgG, Virion/Serion *Coxiella burnetii* Phase I IgA, Virion/Serion *Coxiella burnetii* Phase II IgG, Virion/Serion *Coxiella burnetii* Phase II IgM;
- for determination of Borrelia IgM/IgG, using kits: Anti-*Borrelia* ELISA (IgM) and Anti-*Borrelia* ELISA (IgG), manufactured by EUROIMMUN AG, Germany;
- for determination of *Rickettsia conorii* IgG/IgM using kits: *Rickettsia conorii* ELISA IgG/IgM (Vircell S.L, Spain).

The parameters of IgG avidity to phase I and II *C. burnetii* antigens (if any) were additionally studied according to the earlier described procedure [22].

The patients' blood serum samples were studied on days 1–2 of presentation, and five patients underwent serological testing in paired sera 14 to 30 days afterward.

All patients were prescribed complete blood count and blood chemistry test, urinalysis, as well as other necessary additional investigations to clarify their condition.

## Results

The screening study revealed the presence of specific antibodies to *C. burnetii* in the serum of 10 patients out of 111 patients screened, with negative PCR results (*C. burnetii* DNA in blood plasma). Seropositive patients included 5 men and 5 women older than 54 years. All patients sought medical advice in May–June 2021 and

reported a history of contact with ticks (one patient removed ticks from a domestic animal). While taking epidemiological anamnesis, it was found that prior to presentation, the patients were in one of the regions close to the Moscow Region (Tula, Yaroslavl, Vladimir, Ryazan regions), and four patients were in the Moscow Region. One patient reported multiple cases of tick sucking prior to 2020.

Five of ten seropositive patients received inpatient treatment. They were admitted to hospital on day 5–30 of the disease with the following referral diagnosis: community-acquired pneumonia (two cases), tick-borne borreliosis (one case), tracheobronchitis (one case), and tick-borne encephalitis (one patient). The main complaints were increased body temperature 37.8 °C to 39 °C and weakness. Three patients had history of arthralgia, and two patients had history of erythema migrans. In two patients, the complete blood count demonstrated moderate thrombocytopenia (up to $130\times10^9$/L) and decreased hemoglobin (up to 105 g/L).

The patients sought outpatient consultation after tick sucking without any active complaints, and only one of them had erythema migrans.

In 6/10 examined patients, blood serum testing revealed both antibodies to Q fever pathogen and arthropod-borne infection antigens: to Rickettsia conorii (one patient), to tick-borne encephalitis virus antigens (two patients), and to Borrelia antigens (three patients). Antibodies to *C. burnetii* alone were found in four patients.

Below are presented clinical cases from our clinical practice that demonstrate the complexity of Q fever verification.

## *Clinical case No. 1*

Patient M., female, 62 years of age, was admitted to the Infectious Diseases Hospital on day 12 of disease. The patient had complaints of fever up to 39 °C, marked weakness, non-productive cough, periodic dizziness, sensation of heaviness in the chest, and shortness of breath on exertion. The patient found a sucking tick in the popliteal space on May 2, 2021, while staying at her summer cottage in the Yaroslavl Region. No testing of the tick for the markers of arthropod-borne infections was conducted; the patient had no erythema. On day 3 after tick sucking, the patient noted fever up to 39 °C. One week before hospitalization, after professional medical advice, the patient received amoxicillin 500 mg, twice daily, with no perceptible effect. Due to persisting high fever, patient M. was admitted to hospital by the ambulance crew with diagnosis: Community-acquired pneumonia, condition post tick sucking.

At admission, the patient's condition at admission was considered to be of moderate severity. No swelling, hemorrhages, or exanthems. On examination, there was a crusty ulcer of 3 mm in diameter, no itching or erythema on the skin in the popliteal space. Peripheral lymph nodes were not palpable. On auscultation, there were no rales in the lungs; vesicular breathing; decreased breath sounds on the left; respiratory rate: 23 per minutes. Arterial blood pressure: 125/85 mm Hg; pulse rate: 80 bpm. The liver and spleen were not palpable. Bowel and bladder functions were within normal.

Complete blood count: WBCs $5.5\times10^9$/L; platelets $344\times10^9$/L; lymphocytes $1.73\times10^9$/L; hemoglobin 113 g/L; RBCs $3.42\times10^{12}$/L. The blood chemistry test found no abnormalities: C-reactive protein 45 mg/L (normal limit: up to 5 mg/L); fibrinogen 6.9 g/L (normal limit: up to 4 g/L). PCR test for pathogens of tick-borne encephalitis, anaplasmosis, coronavirus infection, type A and B influenza: negative. No IgG and IgM to *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* were detected. IgG avidity to cytomegalovirus: 84 % (highly avid, postinfectious).

Chest computed tomography (CT) as of May 16, 2021, showed a pattern of bilateral interstitial pneumonia with primary involvement of the left lung. At hospital, the patient was prescribed background intravenous detoxification and antibiotic therapy with ceftriaxone 1 g twice daily, parenterally.

Tests for markers of tick-borne infections, including Q fever, were conducted for epidemiological indications (tick sucking). Molecular genetic markers of pathogens of borreliosis, tick-borne encephalitis, tick-borne rickettsiosis, human granulocytic anaplasmosis, human monocytic ehrlichiosis, Q fever were not detected.

The blood serum test as of May 18, 2021, found anti-Borrelia IgM, cut-off index (COI) = 2.5 (positive ELISA at COI > 1.1), Borrelia IgG: negative. ELISA using test kits manufactured by Vircell S.L found phase II *C. burnetii* IgM in the titer of 1 : 100, in the absence of specific IgG. The second sample tested two weeks after the first sampling revealed the following: Borrelia IgM, COI = 2.9: positive; Borrelia IgG: negative; phase II *C. burnetii* IgM: not detected; phase II *C. burnetii* IgG: positive; final positive titer: 1 : 200. IgG avidity index to phase II *C. burnetii* was 32.2 % (low-avid antibodies), indicative of recent infection.

Blood serum samples were additionally tested by ELISA using the test-systems, allowing for differential detection of antibodies of different classes to phase I and II *C. burnetii* antigens, manufactured by Virion/Serion Institute. Tests of the first and second samples of blood serum from patient M. did not find phase I *C. burnetii* IgG/IgA; however, the test of the first sample revealed phase II *C. burnetii* IgM with optical density (OD) = 0.897 AU (positive ELISA result at OD > 0.680 AU). After treatment initiation, the second blood serum

sample showed a decrease in OD to controversial (borderline) result for IgM. Moreover, the second sample demonstrated an increase in OD signals compared to the first sample while testing for phase II *C. burnetii* IgG to positive (titer 1 : 200). The obtained laboratory findings were in favor of recent co-infection (ixodic tick-borne borreliosis + Q fever).

After treatment, the patient was discharged in satisfactory condition under supervision of the infectious disease physician with recommendations to conduct dynamic testing for specific antibodies to *C. burnetii* for a long time, as well as other investigations.

This clinical case demonstrates the difficulty of Q fever (co-infection) diagnosis without specific laboratory tests.

## *Clinical case No. 2*

On April 13, 2021, patient K., 71 years of age, was admitted to the Infectious Diseases Hospital by the ambulance crew with complaints of dry cough and pyretic fever for one-month, preliminary diagnosis: acute respiratory viral infection, tracheobronchitis, unspecified fever. The patient considered himself to have been ill since March 15, 2021, when the body temperature increased to 39 °C. Earlier, the patient received inpatient treatment for diagnosis: exacerbation of chronic prostatitis and was discharged with improvement; however, he had low-grade fever and complained of lower back pain irradiating to the right hip joint and thigh. On April 5, 2021, the patient consulted an outpatient physician with complaints of fever up to 39 °C, cough, weakness, and lower back pain. Outpatient treatment with levofloxacin, arbidol, and Lasolvan® provided no observable effect. During treatment, the patient underwent chest tomography and PCR for SARS-CoV-2 twice with negative results.

According to the patient's life history: chronic coronary heart disease, functional class 3 angina pectoris, grade 2 hypertension disease, atherosclerosis of aorta and cerebral vessels, chronic pyelonephritis, chronic bronchitis, duodenal ulcer, liver fibrosis. In 2013, the patient received inpatient treatment for spinal injury, and has had lower back pain since then. In 2014, the patient received inpatient treatment in the TB hospital with a diagnosis of nonspecific osteomyelitis; however, no data suggestive of tuberculosis infection were obtained. During several years prior to presentation to the Infectious Diseases Hospital, the patient removed ticks while staying at his summer cottage in the Vladimir Region.

At admission, the patient's condition was considered to be of moderate severity. Body temperature: 38.7 °C. Dry rales in the lungs; heart sounds were muffled and rhythmic; no peripheral edema or hemorrhages. Respiratory rate: 18 per minute; blood pressure: 130/80 mm Hg. Peripheral lymph nodes were not palpable. No signs of scratching or bites. The abdomen was soft on palpation and non-tender in all regions. On palpation, the enlarged dense liver protruded below the costal margin for 4 cm; the spleen was enlarged. Formed, regular stool.

Taking into account the presence of leukocyturia, erythrocyturia, bacteriuria in the urinalysis, urinary tract infection was suspected and antibiotic therapy with ceftriaxone 1 g twice daily intramuscularly and probiotics was prescribed. The body temperature returned to normal on day 2 of the patient's stay in hospital. The complete blood count showed moderate thrombocytopenia ($121\times10^9$/L); the blood chemistry test demonstrated increased alkaline phosphatase activity (240 U/L) and C-reactive protein (15 mg/L). The electrocardiography examination found left bundle branch block.

Based on the combination of life history and investigation data, it was decided to perform additional blood serum testing for specific markers of arthropod-borne infections, including Q fever. The PCR test did not reveal genetic markers of pathogens of borreliosis, tick-borne encephalitis, tick-borne rickettsiosis, human granulocytic anaplasmosis, human monocytic ehrlichiosis, Q fever. However, the blood serum testing by the ELISA method detected phase II *C. burnetii* IgG (OD = 1.121 AU, positive result: >0.78 AU) in the absence of phase II *C. burnetii* IgM. Final positive titer: 1 : 500. IgG avidity to phase II *C. burnetii* was 76% (highly avid). To clarify the stage of Q fever, an additional test for phase I *C. burnetii* IgG/IgA was conducted. The blood serum test found phase I *C. burnetii* IgA with OD = 1.500 AU (positive result: >1.081 AU), titer 1 : 800. The obtained laboratory data were in favor of probable chronic Q fever. On April 19, 2021, the patient was discharged in satisfactory condition under supervision of the infectious disease physician with recommendations to conduct dynamic testing for specific antibodies for a long time, as well as other investigations.

This clinical case demonstrates the difficulty of recognizing Q fever in chronic stage without specific laboratory tests, and the lack of physician suspicion of Q Fever, as in the clinical case described above.

## *Clinical case No. 3*

On June 2, 2021, patient E., 55 years of age, presented to the Consulting and Outpatient Department of the Infectious Diseases Hospital, Moscow due to Borrelia DNA detected in the tick, which the patient removed on May 6, 2021. No testing of the tick for *C. burnetii* and Rickettsia DNA was conducted. At presentation, the patient had no complaints. The tick sucking took place in the Vladimir Region. The patient did not receive medical

therapy. According to medical history, the patient was earlier treated for chronic HCV infection. When examining the tick sucking site in the right axillary space, no erythema was found. Due to detection of Borrelia DNA in the tick, patient E. was prescribed antibiotic therapy with amoxicillin/clavulonic acid at a dose of 875/125 mg twice daily for 10 days.

Taking into account the fact of tick sucking, patient E. underwent additional blood plasma/serum tests for the presence of markers of tick-borne infections, including Q fever. The blood test did not reveal genetic markers of pathogens of borreliosis, tick-borne encephalitis, tick-borne rickettsiosis, human granulocytic anaplasmosis, human monocytic ehrlichiosis, Q fever. At the same time, the blood serum test by the ELISA method using the test system, manufactured by Vircell, as of June 2, 2021, revealed phase II *C. burnetii* IgG, COI = 13.6; final positive titer: 1 : 500. The paired blood serum sample test, conducted two weeks after the first sample, showed a slight decrease in COI to 11.1; final positive serum titer: 1 : 500. Phase I *C. burnetii* IgG were determined in both samples: at blood serum dilution 1 : 500, signal OD (first sample) was 0.948 AU (cut off = 0.670); in the second sample, OD = 0.866. Phase I *C. burnetii* IgA and specific IgM to the pathogen were not detected.

In the first sample, IgG avidity to phase I *C. burnetii* was 87 %, phase II *C. burnetii* IgG was 74.5 %. Two weeks later, the avidity values were almost the same: 85.8 % and 77.2 %, respectively.

The high level of class G antibodies to the pathogen in phase I state and highly avid IgG (with an excess of phase I versus phase II IgG avidity) were in favor of long-term infection with *C. burnetii* in the patient, but this fact was established for the first time. According to the combination of laboratory data, the chronic stage of Q fever in patient E. cannot be ruled out.

The patient was recommended serological monitoring of antibodies to *C. burnetii* and other necessary investigations to prevent complications.

## Discussion

The absence of pathognomonic clinical signs of Q fever and the frequent subclinical course of the disease leads to the fact that it remains undiagnosed in the majority of cases. At the same time, *C. burnetii* infection can lead to severe complications, sometimes fatal for the patient. The disease etiology cannot be proven without specific laboratory diagnostic methods. The laboratory examination is indicated to individuals based on the epidemiological anamnesis (work in animal breeding; husbandry and care of cattle and small ruminants, poultry; consumption of raw milk, dairy and meat products that have not been sufficiently processed); patients with fever, intoxication syndrome, respiratory involvement, hepatomegaly, jaundice syndrome, exanthems, hemostasis, gas exchange disorders, and complications [16]. This list of clinical signs can be supplemented by the observations of other leading researchers in the disease area, especially those who managed patients during and after the largest outbreak of Q fever in the Netherlands in 2007–2010, when the number of infected people exceeded 4,000 [23]. Due to the high cost of necessary diagnostic kits, in practice, they are often limited either to detection of antibodies (most often without differential assessment of immunoglobulins to Coxiellae in two phase states) or to detection of pathogen DNA.

The given study demonstrated that Q fever can be found in the Moscow and neighboring regions; however, its diagnosis is complicated. The PCR test did not reveal pathogen DNA in any of the tested blood plasma samples. In most cases, blood sampling from the patients who were seropositive to *C. burnetii* was performed after the start of antibiotic therapy or long after the disease onset. Therefore, when making a decision on the absence of infection, we did not consider a negative PCR to be definitive. The chronic phase is as important to be recognized as the acute phase, since the risk of life-threatening complications increases with disease progression. Therefore, a two-stage study was conducted: the first stage included serological screening for phase II *C. burnetii* IgG/IgM, and if a positive result was obtained, the study was supplemented by detection of phase I *C. burnetii* IgA/IgG, as well as assessment of antibody avidity. Q fever was serologically confirmed in 10 patients, since there was an opportunity to conduct thorough study of the clinical material in the presence of relevant diagnostic kits. The assessment of IgG avidity contributed to the disease stage clarification.

In the described clinical case No. 1, primary acute Q fever (concomitant infection of borreliosis) was confirmed in laboratory settings by seroconversion of antibodies to phase *II C. burnetii* antigens. Notably, there were specific IgM detected in the first sample, while a switch in the immunoglobulin synthesis from IgM to IgG was observed in the second sample. Low-avid IgG were indicative of a recent infection with Coxiellae, which was likely to have occurred via a vector-borne pathway. In this case, the main clinical symptoms included fever, weakness, and signs of pneumonia.

It is necessary to pay special attention to the elderly patients and those complaining of long-term fever in order to rule out *Coxiella burnetii* infection. Collection of complete medical history of the current condition, past medical history, and epidemiological data gains special importance In clinical case No. 2, one of the possible clinical manifestations of chronic Q fever was described, probably during exacerbation, taking into

account aggravation of the patient's condition and current fever. This has found laboratory confirmation in the form of increased levels of antibodies to the lipopolysaccharide complex of phase I *C. burnetii,* which quite often correlates to the development of Q fever complications, especially in the cardiovascular system [21]. Specific IgGs were considered to be highly avid, which further confirmed long-term infection that had not been previously recognized in time and probably caused a number of complications.

Clinical case No. 3 is a good example of obliterated and unpronounced signs of Q fever. The presence of phase I *C. burnetii* IgG and highly avid IgG were suggestive of long-term infection in the patient. This fact was established occasionally.

## Conclusions

In some cases, the aggregate of clinical and epidemiological data did not allow suspecting Q fever in a patient. The disease etiology cannot be established without specific laboratory diagnostic methods. However, the laboratory diagnosis of Q fever is also accompanied by certain difficulties, since a negative PCR for *C. burnetii* DNA does not allow ruling out infection in a patient. Moreover, regardless of the disease stage, the leading expert working groups on Q fever recommend long-term serological monitoring for up to 5 years to prevent severe complications and relapses [11]. In this regard, the importance of additional serological studies is in no doubt: all patients with suspected Q fever and those previously diagnosed should be tested for the presence of specific antibodies to phase I and II *C. burnetii.*

The study of the serological profile with differential assessment of titers (levels) of antibodies of different classes to the pathogen and their avidity can give the treating physician a lot of valuable information about the infection course. In our opinion, expanded studies for markers of Q fever (pathogen DNA; titer of phase I and II *C. burnetii* IgA, IgM, IgG; avidity index) in the group of individuals affected by the tick bite, as well as among patients with fever and unknown disease etiology, are promising. The data from the study will allow improving the diagnostic algorithm of Q fever and patient management strategy in cases of suspected Q fever.

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