International Journal of General
Medicine and Pharmacy (IJGMP)
ISSN(P): 2319–3999; ISSN(E): 2319–4006
Vol. 9, Issue 1, Dec–Jan 2020; 1–16

International Academy of Science,
Engineering and Technology
Connecting Researchers; Nurturing Innovations

COXIELOSIS HEALTH PROBLEM IN HUMAN AND VETERINARY PATHOLOGY IN MONTENEGRO

Bogdanka Andric[1], Stefan Kecina[2], Milan Jovanovic[3], Brankica Dupanovic[4], Snezana Dragas[5] & Davor Korunic[6]

[1,2,3, 4,5,] Research Scholar, Infectious Diseases Clinic Faculty of Medicine University of Montenegro, Podgorica, Montenegro

[6]Research Scholar, Private Veterinary Practice, Grandov, Bijelo Polje, Podgorica, Montenegro

ABSTRACT

Coxielosis (Q fever) is recording in all geographical areas and climatic zones, where the natural conditions and sources of the causative agent C.b. exists. The Mediterranean area is rich in natural resources for the existence of C.b., which is classified as a dangerous pathogen. C.b. is the cause of acute and chronic infections. In humans, 60% of infections with C.b. has subclinical flow. Clinically manifest cases are variable symptoms. From self-limiting flu-like syndromes' pneumonia, this is one of the most frequent manifestations of the acute phase of disease. Hepatitis with or without jaundice. Endocarditis is one of the extremely dangerous manifestations of coxielosis that endanger patients' lives, usually in the chronic phase of infection as well as the spectrum of CNS manifestations. Other organs are less commonly affected. Immune deficient patients present candidates for the development of extremely severe, chronic forms of the disease.

In Montenegro, the first case of Q fever in human pathology has been etiologically confirming in 1994/1995. In 2003, another 10 cases with coxielosis have been detected. In the same period, animal prevalence surveys showed a 0.29% representation of the total livestock (sheep) stock in Montenegro, with objective assumptions that the problem was of a much wider scale..

During the period (1996–2017), 2450 sera of patients were tested for co-infective forms of Lyme borreliosis. In the group of epidemiologically conditioned co-infection, the richest agents were most frequently registering, among which C.b. in 126 cases. During 2018/2019, 12 patients with Q fever were treated in clinic for infectious disease in Podgorica. In the same period, veterinarians examined 251 sera of domestic animals and found dominant disease of cattle coxellosis in 210 cases, 54 in sheep and only 5 diseased goats. Acute cases were treated with doxycycline for at least –two to three weeks. Chronic infections are treated with doxycycline +hydroxychloroquine until withdrawal of symptoms. For endocarditis, lifelong treatment is recommended because relapses have been observed during discontinuation of treatment.

***KEYWORDS**: Coxielosis; Multisystemic; Acute; Chronic Disease*

Article History

***Received:** 22 Oct 2019 | **Revised:** 19 Nov 2019 | **Accepted:** 22 Nov 2019*

INTRODUCTION

Coxielosis (Q fever) is zoonosis widespread worldwide. It has been registered in all geographical areas and climate zones, where the natural conditions and sources of the causative agents Coxiella burnetii (C.b.) exist. The registration of this

disease has considerable variations in its prevalence. The diseases prevalent among humans and animals worldwide depend on the medical and scientific proof of zoonosis.(1)

The history of Q fever dates from the period 1920–1930s and identification of the new type of bacteria. In 1937 in Queensland (Australia), Derrick described febrile disease which he called "Query fever" (2). Then, Burnet and Freem had isolates, the causative agents from blood and urine of Australian patients and based on sequencing 16S rRNA gene and determinants of phylogenetic relations, they classified it into the group of rikettsial agents (3). Coxielae belonged to subgroup 2 proteobacteria and rickettsiae to a subgroup 1a. Davis and Cox simultaneously isolated agents from the tick in Montana, USA and named it rikettsia diasporica because of their ability to pass through pores of filters (4). Later, it was renamed into C.b. in honor of the first group of researchers.

Coxiella could hardly be examined because of its impossibility of reproduction outside the host. The technique for its laboratory cultivation has been adapted since 2009 (5, 6). C.b. belongs to a group of dangerous pathogens (7, 8, 9, 10). In the past, it was a part of the program about biological weapons, categorized into bioterrorist agents of group "B", because of its low contagious doses, stability in external environment and ability of aerosol dispersion (11, 12, 13).

C.b. is Gram-negative, pleomorphic coccobacillus member of bacterial family coxiellaceae. It has DNA and RNA. Using the method *Multispacer Sequence Typing* (MTS) there were over 30 of its genotypes discovered (14, 15). Although C.b. is still classified and considered as rickettsiae, new researches suggest its phylogenetic connection with bacteria: Legionella, Francisella, Pseudomonas and other Gama-proteobacteria (16). (Figure 1)

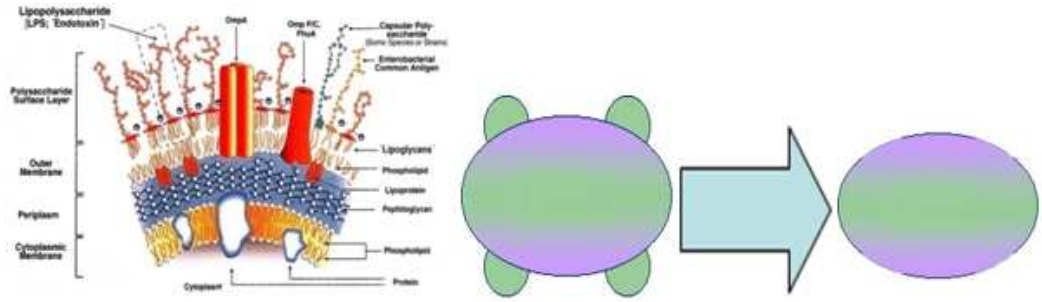

Figure 1: Complexity of C.B. Structure (A), C.B. Stages Variations (B)

C.b. shows up in three different forms: large cell variant (LCV) is vegetative form, which exists in infected cells and cellular cultures. Small cell variant (SCV) are extracellular, highly infectious forms; in high concentration (109 ID50/g) they exist in animal placental tissue and amnion liquid, milk, urine and feces. Very resistant, they can survive and persist in external environment for years. After an acute infection, C.b. can localize in inflammation granuloma, from where it can be reactivated in conditions of nonspecific and specific immunodeficiency. Third variant are slow cells (SLP), very contagious and very robust about environment conditions (17).

In sensitive host tissues, during the growth C.b. goes through stage variations, stage I and stage II (18). Variations of stage II signify acute infection and stage I chronic infection (19, 20). Lipopolysaccharides (LPS) are the most significant determinants of C.b. pathogenicity. Also, lymphoproliferative prevalence or mitogen T-cell response in persons who have C.b. antigens has been proven (21). Four different types of plasmid or chromosomally integrated plasmid-homologous sequences could be founded in both C.b. stages. Every bacterial cell has only one of four plasmids, which contains around 2% of genetic information. Based on nucleic acid analysis of sequences of different gene regions, including plasmids,

classification of C.b. into genotypes or gene groups (if several genotypes are grouped together), has been suggested by many authors. Individual genotypes show only partial synchronism, but enough to cause the disease (22).

C.b. belongs to bacteria which use IVB system for excretion, known as *Icm/Dot (intracellular multiplication/because of gene defect for merger with organelle).* In infected organism of the host, they trigger production of proteins marked as Ank proteins (effectors), which increase the ability of bacteria to survive inside the cells of a host. At Legionellae pneumophila, which uses the same system of secretion and injection of Ank proteins, the survival is improved because Ank proteins interfere with fusion of vacuoles that contain bacteria with endosomes of degradation of host's cells (23, 24).

Coxiellae may classify into geno-groups I–VI with individual groups, which contain between 1 and 15 MST (genotypes). It was considered that the part of diversity originated because of mutations that appear in co-circulatory routes of C.b. Pathogenicity and virulence varies between different geno-groups of C.b. It is proving that those MST-8 genotypes, whose reservoir are goats, is the causer of people's chronic infection and often endocarditis (25).

C.b. uses two main patterns of transfer to hosts. In one, it circles among wild animals and their ectoparasites, mostly ticks. Ixodes and argasidae ticks, except vector function, may also be reservoirs of ageneses, considering their sexual cycle, which allows maintaining C.b. through more tick generations (26). The second way of transfer appears among domestic cuds (27). (Figure 2)

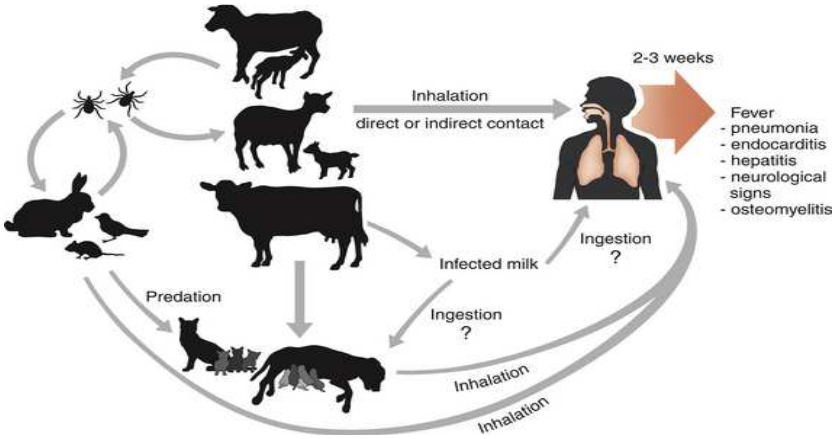

Figure 2: Two Main Patterns of C.B. Transfer: at Wild Animals, where Ticks have Primary Significance. the Other Way of Transfer Happens at Domestic Cuds, Apart from Cycle of Wild Animals.

Spectrum of C.b. hosts includes different kinds of wild and domestic mammals, arthropods and birds. The disease is enzootic in most areas, where cattle are grown, especially sheep and goats. Seroprevalence studies on farms in many countries in European and American continent show large variations. Seroprevalence at professionally exposed individuals (veterinarians, cattlemen, farmers and butchers) is also high (27).

As regards people, the biggest risk for C.b. transfer is created when aerosol and dust are inhaled. There is also the possibility of alimentary transfer or by direct contact with body fluids and organs of infected animals (meat, skin, milk, urine, feces and placenta) is also high (28). There is also the possibility of transfer by blood and blood derivates (29), sexually, vertically from mother to fetus (30, 32). Newer researches show the possibility of interhuman transfer of infective agents in family conditions, but it is still insufficiently defined (31).

Over 70 kinds of ticks could transfer the disease among domestic and wild animals, which is important for maintaining C.b. in animal populations, although it is considered they do not have significant epidemiological importance in transferring the disease onto the man, the possibility of transfer through stitch and feces of ticks has been proven (26). Sheep, goats and cattle are the most significant reservoirs and main sources for human infection. Cats, dogs, rabbits, wild animals and ducks have also been identified as sources of infection.

Q fever is registered at laboratory workers in medical institutions in which latent infected sheep were used for research. As regards humans, sexual transfer of C.b. from husband, in later stages of recovery from professionally acquired acute Q fever, onto wife. The DNA of C.b. was proven in husband's sperm samples with PCR method, which is a valid proof of disease transfer route. Vertical transfer from mother to a child has also been registered (32).

C.b. is the cause of subclinical, acute and chronic infections. It is experimentally proven that it was enough to inhale 1-10 microorganisms for infection in 50% of respondents. This extremely low-infective dose categorizes C.b. among dangerous pathogens (33).

For people, incubation period for coxiellosis is 14–60 days (20 days in average). Clinical presentation is variable. In 50% of infected individuals, it goes subclinical. Speaking about manifest infections, it goes from self-limiting disease similar to flu, to a spectrum of variable clinic manifestations, which can engage many tissues and organs. The most frequent acute stage manifestations are pneumonia and anicteric, rarely icteric hepatitis. Unlike the animals, coxiellosis in humans is rarely connected with abortions, but it had been found on women (34). Besides vertical transfer from mother to a child, studies of late 1940s showed that after the primary infection, C.b. was transferred sexually and it causes changes in vulva and cervix as well as purulent **orchiepididymitis** in men. Newer researches indicate possibility of other ways of inter-humans causative agent transfer. The third variant is perfidious chronic disease.(Table 1).

Table 1: Clinical Manifestations of Infections with C. Burnet Ii

Akutne	Hronične	Druge
-Status febrile 2 – 14 day with high febricity, malaise, mialgiae, artralgiae -Pneumonia	Granulomatous hepatitis Endocarditis Osteomyelitis Pneumonitis Interstitial lung fibrosis Hepatitis (with jaundice or non jaundice) Purulent orchitis Disorders of the vulva, cervix uteri, ovaries.	Q fever in immune-deficient patients Q fever in children CNS manifestations - Encephalitis - Aseptic meningitis - Dementia - Extrapyramidal disturbances

The most frequent clinical manifestations of acute Q fever include

In Acute Phases

- -Febrile state 2–14 days with high temperature, fatigue, myalgia and arthralgia
- -Pneumonia

In Chronic Phases

Granulomatous hepatitis, Endocarditis, Osteomyelitis, Pneumonitis, Interstitial fibrosis of lungs, purulent orchitis, Changes of vulva and cervix,

Other Manifestations

Q on the immune deficient, Q fever on children,

CNS manifestations

- Encephalitis
- Aseptic meningitis
- Dementia
- Extrapyramidal disorders

Extremely difficult, chronic forms of disease often appear on persons with immunodeficiency. Patients who have artificial heart valves are specifically under the risk for endocarditis development when HIV, hard and fatal forms of infections engage many tissues and organs.

Co-infections are also a predisposing factor for development of difficult and chronic forms of disease.

Besides endocarditis, osteomyelitis and other heavy disorders, fatigue syndrome is registered in 20% of patients and is the most common result after an acute infection (35, 36, 37, 38).

Stage variations of causative agent's condition and stage variations of specific antibodies (At) on people. At II stage signify acute infection and I stage signify chronic. Indirect immune-fluorescence (IIF) represents choice method for routine diagnosis of Q fever. Microfluorescence test has been considered as a reference standard since 1983 (39). The test is positive after one to two weeks after the first symptom of disease. The method of IIF titers $\geq$ 1:50 for IgM and $\geq$ 1:200 for IgG. They are considered as positive on acute Q fever and titers $\geq$ 1:800 for IgG are considered positive on chronic Q fever (40). Seroconversion, apropos quadruple increase of titers during the infection is considered to be positive on acute or reactivated infection detection.

ELISA and the complement attachment test (CFA): they are also used for routine diagnosis. ELISA has been used for identification of diseased blood donors in Netherlands and reactive samples were further analyzed with PCR-method (41). CFA is usually used for early studies of prevalence (109).

With a Western blot method, 15 antigens with molecular weight of 20–160 kDa can be discovered on C.b. strip. Seven to ten different antigens could be colored with serums from acute phase of infection and 12–15 antigens can be colored with serums from chronic phase (42). (Figure 3)

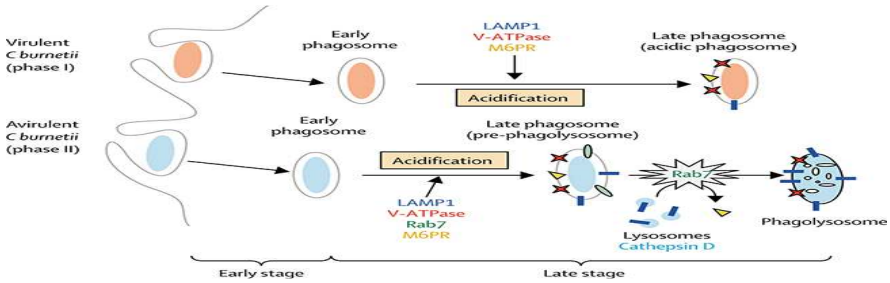

Figure 2: Diagnosis of Acute and Chronic Stage of Q Fever.

Polymerase Chain Reaction (PCR) is used for detection of nucleic acid of C.b. With this method, C.b. can be detected in blood 2two weeks after transmission (41). For genotyping C.b., we used reliable PCR methods of detection (9, 18, 22).

Considering the fact that C.b. grows in microphages and monocytes, it may cause temporary bacteremia and through blood flow and lymph flow, it can reach distant spots in organism, which confirms its presence in different body fluids (34, 42, 43, 44, 45). In chronic infections of the walls of the blood vessels, its reactivation can also be expected. It is considered that most of the infections with C.b. (> 60%) with or without fever are self-limiting and it comes to a spontaneous recovery, but newer researches indicate on the possibility of C.b. to reintegrate latent infections, from which it can reactivate and allow other hypothesis. In case of clinically manifested infections, we can expect mortality rate of 2%, depending on the time of starting antibiotic treatment in regard to beginning of disease and depending on the immune status and the age of infected individuals.

Tetracycline and doxycycline therapy reduces symptomatic duration of disease and reduces the possibility of infection progression. Doxycycline is a medicine of choice. Other efficient treatments involve ampicillin, chloramphenicol and fluoroquinolones and in some cases, rifampicin in combination with doxycycline or ciprofloxacin (46). Acute infections should be treated for at least two weeks. Chronic infection is treated until symptoms are retreated, preferably with doxycycline in combination with hydroxychloroquine. Therapy treatment usually lasts around three years. Some authors recommend treatment for life in case of endocarditis since sub-reactivations (relapses) are spotted during the treatment break. Newer alternatives for antibiotic treatment include tigecyline and clarithromycin (47). Vaccination with Q-VAX vaccine (CSL) is efficient preventive to stop disease occurrence. It is recommended to use it for vaccination of professionally disposed persons. Speaking of sheep, initial use has not reduced infection, but it did reduce excretion of C.b. 92–98% (48).

Material and Methodology

In Montenegro, considering the natural resources, Q fever surely has longer history, but there were not any extensive examination of its prevalence and significance done, especially in human pathology. Veterinary examinations led the way and indicated its potential importance (49). First case of Q fever in human pathology was etiologically verified in 1995/1996 by IIF method. At the end of 2003, it has been detected on 10 more diseased people. At the same period, researches of disease prevalence among animals showed representation of 0, 29% concerning total cattle (sheep) fund in Montenegro, with objective presumptions that the problem has pretty wider proportions (49).

Between 2007 and 2016, incidence of Q fever in Montenegro progressed in the range of 0-1, 1/1,00,000 citizens (49). Between 2006 and 2007, Q fever on cattle and sheep was detected on both sides of the border (between Serbia and Montenegro).

During the period of 1996–2017, 2450 serums of patients were examined for co-infection forms of Lyme borreliosis (LB). On that occasion, representative involvement of rickety ageneses has been affirming and C.b. was detected on 126 respondents. During 2018/19, 12 patients with confirmed diagnosis of Q fever were treated in infective diseases clinic in Podgorica, note that at least 50% of human infections with C.b. go subclinical for what they are not recorded. For etiological Dg, methods that have been used are IIF, ELISA, WB, PCR. Additional methods: marrow bone biopsy, peripheral blood smear, x-ray diagnosis, hematological diagnosis, US, CT, NMR, EMG, EKG, echocardiography, etc. Retrospective analysis of characteristics in 149 cases of Q fever indicated the following characteristics:

RESULTS

During our examination of Q fever on 149 cases, in clinical presentation, symptoms of general infective syndrome dominated, lasting 1–4 weeks with high temperature, fever, perspiration, nausea, vomiting, diarrhea, headache, resistant to

analgesics. Respiratory tract symptoms (pneumonia with enlargement of Hilary lymph nodes) have been registered on 138 respondents. In four cases, besides respiratory syndromes, acute hepatitis was manifested. In three cases, acute hepatitis of non-respiratory syndromes with splenomegaly. In one case, of an older woman, aged 72, there was a manifestation of chronic infection with endocarditis. Her daughter aged 28 had also had difficult chronic infection, which manifested as hard lobe, recurrent pneumonia, headache and chronic fatigue (both patients coming from the same household of four members). The householder is a veterinarian and all the members of his household were diseased of Q fever (family epidemic). (Table 2). According to veterinary examination, only one bovine in their stable was detected of Q fever.

Table 2: Representation of Clinical Manifestations on our Patients

Representation of Multisystemic Discorders N=149		
General infective symptoms	149	100%
CNS (headache resistant to analgesics)	37	
Cardiovascular symptoms	1	
Respiratory symptoms	138	
Gastrointestinal symptoms	10	
Hepatitis	7	
Urogenital symptoms	2	

During 2018, monthly report about confirmed cases of Q fever on domestic animals in Montenegro has been presented (Table 3). Based on presented results, 251 domestic animals were registered as Q fever disease during 2018. The most significant is representation of large cattle – 210 cases, 54 cases of sheep and the goats were represented with only five diseased animals.

Table 3: Confirmed Cases of Q Fever in Domestic Animals in Montenegro, During 2018

Monthly Report about Confirmed cases of Q Fever on Animals in Montenegro During 2018				
Month	**Municipality**	**Kind of Animals**	**Number of Households**	**Number of Disease Registered**
January	Nikšić	cattle	1	2
February	Kolašin	goats	1	3
	Nikšić	cattle	1	13
March	Nikšić	cattle	1	4
		sheep		1
	Plužine	Cattle	1	1
April	Nema potvrđenih			
May	Podgorica	catlle	1	4
	Nikšić	sheep	1	1
June	Podgorica	sheep	1	56
	Danilovgrad	koze	2	2
July	Bijelo Polje	sheep	2	25
	Podgorica	sheep	2	73
August	Nikšić	Cattle	1	8
	Danilovgrad	cattle	1	7
September	Nikšić	Cattle	1	3
	Danilovgrad	Cattle	1	1
	Podgorica	Sheep	1	15
	Plužine	Sheep	1	30
Oktober	Nikšić	Cattle	1	3
	Plužine	sheep	1	3
November	No registered cases			
December	Plužine	sheep	1	6
Total number of registered Q fever cases on domestic animals in 2018				**251**

DISCUSSIONS

In Europe, the Mediterranean area abounds with ideal natural resources for existence of Q fever causative agents. Data from medical literature and numerous researches warn us on its presence in many countries and also in in human and veterinary pathology (49, 50, 51, 52). First case of Q fever in southeast Europe, during World War II, had been described as an epidemic phenomenon of an unknown limit, named "Balkan flu" in countries of the Mediterranean (among German soldiers in Balkan and American soldiers in Italy). This disease was identified as Q fever in 1946, after isolation and identification of C.b. from these epidemics.

Q fever epidemic had been registered in 1949 in South Banat (Vojvodina, ex Yugoslavia). Since then, sporadic cases of this disease and epidemic have been repeatedly registered in all parts of Balkan countries. Extensive examinations of Vuksic and his associates in 1954 defined endemoepidemic character and significance of the disease, establishing seroprevalence of 39% on sheep in Sandzak area.

Dejan Lausevic's veterinary researches during 2002/2003 in Montenegro area indicated representation 0, 29% in total cattle (sheep) fund in Montenegro with objective presumptions that the problem is of significantly wider scale (53). Based on veterinarian researches over a period of time, 2007–2016, incidence of Q fever in Montenegro progressed within a range of 0–1,1/1,00,000 citizens (53).

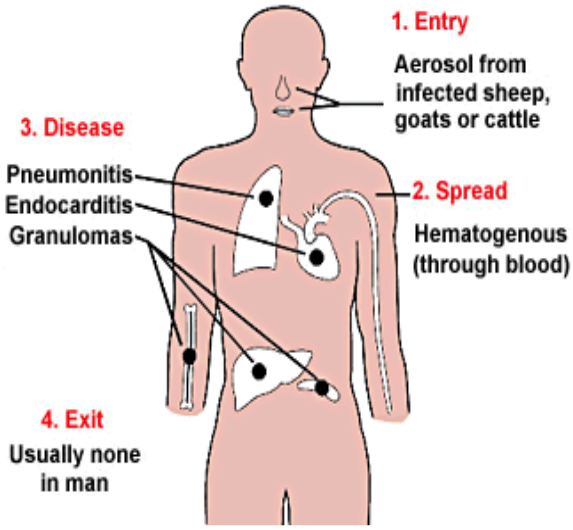

Figure 4: Nonspecific Clinical Symptoms During Q Fever, Span Different Tissues and Organs

Incubation period for Q fever amounts 14 - 60 days, average 20 days. In humans and animals, C.b. can survive in a new host and lead to acute and chronic manifestations. In clinic picture, non-specific symptoms: fever, perspiration, nausea, vomiting, diarrhea, extreme fatigue appeared in 5–20% of patients as manifestation after the fever. Rarely, some other organs may be infected. (Figure 4) In chronic phases, among other chronic manifestations of Q fever, they are particularly significant: subacute endocarditis, chronic hepatitis, bone marrow and joint lesions, post-infection fatigue syndrome. CNS lesion. C.b. can establish latency. If late reactivation occurs in late pregnancy, microorganism by vertical transmission can damage the fetus or lead to preterm birth. C.b. is also mentioned as possible causative agent of tumors and Non-Hodgkin's lymphoma (54, 55).

Agents can be present in blood flow or lymph flow during acute stage, considering the fact that C.b. grows into macrophages and monocytes and during the temporary bacteremia, it could spread to the most distant spots in organism. The proof is indicated in its detection in different tissues (CNS, kidneys, spleen, lymph nodes, etc.) and body fluids (cerebrospinal fluid, urine, bronchopulmonary secretion, milk, vaginal secretion and sperm, etc.) (56, 57, 58). In chronic infections of blood vessel walls, it should be expected that C.b. can be temporarily released and it can cause difficult disorders (59, 60, 61, 62). In case of clinical infections' manifestation, we should expect mortality rate of 2% depending on the moment of starting with the antibiotic treatment in regard to beginning of the disease, age and immunological status of the infected individual.

Pneumonia is the most frequent clinical manifestation of Q fever (acute stage, in 1–2% of patients). The most common is lobar pneumonia with high fever. In our cases, what dominated was interstitial pneumonia, rarely lobar segmental or non-segmental pneumonia (10% of patients). Speaking of our diseased, we have not registered fast onset pneumonia, which clinically remind of pneumonia caused by Legionella pneumophila. X-ray diagnosis shows resulting granulomas as multiple round shadows (Figure 5). Its complications can lead to atelectasis and enlargement of hilar lymph nodes. In pregnant women, it can be transmitted to the fetus and cause severe malformations (65,66), antiphospholipid antibody syndrome with valvular vegetation is described in acute phases of Q fever (67).

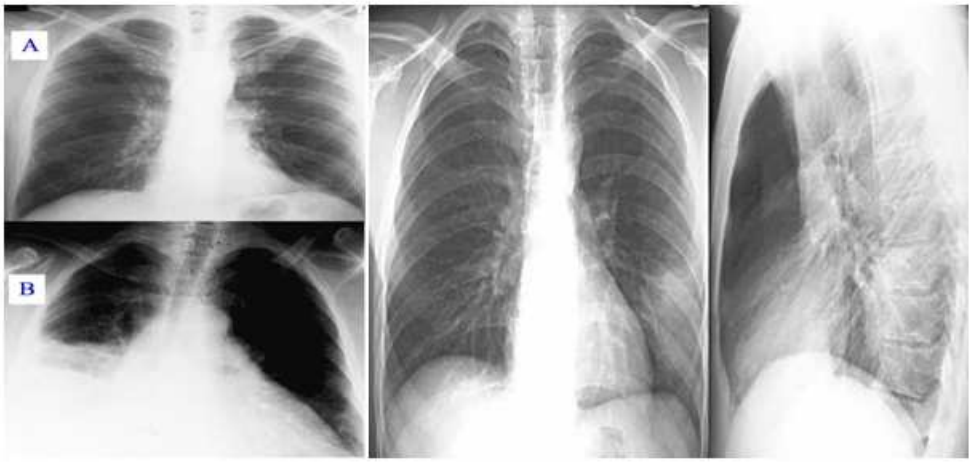

Figure 5: Pneumonia is the Most Frequent Clinical Manifestation of Q Fever's Acute Stage

Studies in the late 1940s showed that after primary infection with C.b., it could be sexually transmitted through interhumans. This possibility is confirmed by the finding of C.b. in the vaginal secretions and sperm (Figure 6 A, B, C).

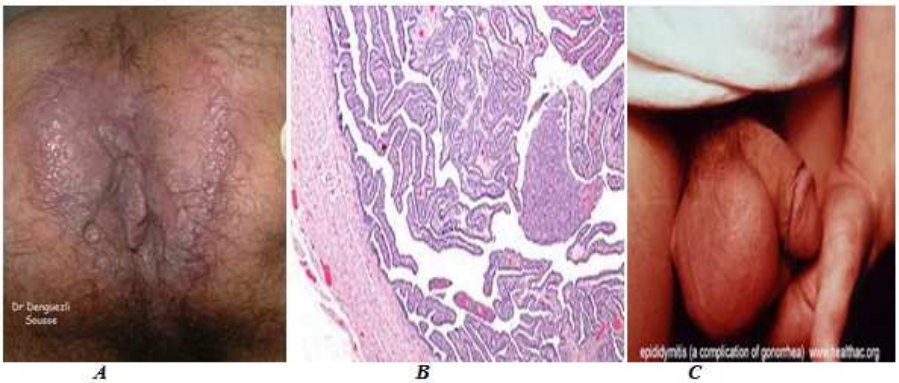

Figure 6: Clinical Changes in the Vulva (A) And Pathohistological Changes in

the Cervix (B) in a Woman as well as Purulent Orchiepididymitis in a Man (C)

It can affect the bone marrow, ovaries and testes. Orchitis is a recognized, common and late complication of acute Q fever in men; the incidence varies from case to case. An example is given of a patient from Australia. CNS are less commonly affected.

Orchitis is a recognized, common and late complication of acute Q fever in men.The incidence varies from case to case. An example is given of a patient from Australia in six cases.

In a group of patients in which we searched for coinfectious forms of LB transferred by a stitch of a tick, C.b. besides bb has been detected in 126 examinees, as coinfective agents. All the cases had hard clinical picture of respiratory symptoms, and in over 45% of cases, chronic flow of the disease was registered.

If Q fever is not treated properly and long enough, C.b. survives for years in granulomas, which are formed in different tissues and organs (63, 64, 65) and it can relapse. Relapses are common and in chronic infections of blood vessels with periodical bacteremia. In cases of acute infection, antibiotic treatment lasts for two to three weeks. Speaking of chronic Q fever, it is recommended to use combined antibiotic therapy for at least 18 months and when it is about endocarditis there are attitudes that recommend therapy for-life.

Endocarditis is categorized among most difficult manifestations of chronic Q fever (61, 69). In these patients, there is often splenomegaly presence and it is often related to anemia and hematuria.

Neurological syndromes during Q fever are represented by a wide range of disorders, from frequent symptomatic headache and meningismus, which was registered in our examinations, to aseptic meningitis, encephalitis, paresthesia, sensory ability disorders, Guillain-Barre syndrome. In cerebrospinal fluid (CSF), we can find mononuclear cells; proteinuria, and rarely can be isolated causative agent of C.b. As regards ocular disorders, C.b. may cause uveitis and retinal damage with impaired vision (66, 67, 68, 69, 70, 71). Abdominal pain and diarrheal syndrome are also represented. In certain cases, differential-diagnostic problem in regard to endocarditis (72).

CONCLUSIONS

Q fever is a very difficult disease of zoonosis that affects animal and human population. Referring to people, small infective dose, resistance in external environment, possibility of aerosol dispersion and wider possibilities of transmission are significant risk factors for people's disease, especially in endemic areas, where cattle have been raised. Newer researches warn on C.b. infective agents host spectrum expansion, which may affect increment of disease prevalence.

It is very hard to set up diagnosis for Q fever, considering the fact that over 50% of infections go subclinical. Symptomatic infections abound with nonspecific symptoms that engage many systems and organs and do not support clinical recognition of the disease, which is the first step for diagnosis and is of most significance for prompt and adequate therapy, prevention of disease progression. Immunological examinations and pathological results that discover persistent C.b. infections, supplemented by knowledge about possibilities of its reactivation are warning factors referring to definition of Q fever as self-limiting disease and stimulus for further research on Q fever demystification.

REFERENCES

1. *Derrick, E. H. (1937): Q fever a new fever entity: clinical features, diagnosis and laboratory investigation. Med. J. Aust.;2:281-199 (Pub Med)*
2. *Burnet, F. M. and Freeman M. (1937): Experimental studies on the virus of Q fever. Med. J. Aust.:2:299–305*
3. *Davis, G. and Cox, H. R., (1938): A filter pressing infectious agent isolated from tick: Isolation from Dermacentor andersoni, reaction in animals, and filtration experiments. Public Health Rep. ;53:2259 – 2267.*
4. *McDade, Joseph, E. (1990): Historical Aspects of Q Fever, in Marrie, Thomas J., Q Fever, Volume I: The Disease, CRC Press, pp. 5–22, ISBN 0-8493-5984-8*
5. *Raoult, D., Marrie, T. and Mege, J. (2005): Natural history and pathophysiology of Q fever. Lancet Infect. Dis.;5: 219–226. [PubMed]*
6. *Arricau-Bouvery, N. and Rodolaksi, A., (2005): Is Q fever an emerging or reemerging zoonosis? Vet. Resp.;36:327–349 (Pub Med)*
7. *Tilburg, J. H. C, Roest, H. J. I., Buffet, S., Nabuurs-Franssen, M. H., Horrevorts, A. M., Raoult, D. and Klaassen, C. H. W.(2012) : Epidemic genotype of Coxiella burnetii among goats, sheep, and humans in the Netherlands. Emerg. Infect. Dis. 2012;18:887–889. [PMC free article] [PubMed]*
8. *McCaul, T. F., and Williams, J. C. (1981): Development cycle of Coxiella burnetii: structure and morphogenesis of vegetative and sporogenic differentiation. J. Bacteriol. 1981; 147burnetii: 1063–1076. [PMC free article] [PubMed]*
9. *Jado, I., Carranza-Rodriguez, C., Barandika, J. F., Toledo, A., Garcia-Amil, C., Serrano, B., Bolaños, M., Gil, H., Escudero, R., Garcia-Pérez, A. L., Olmeda, A. S., Astobiza, I., Lobo, B., Rodriguez-Vargas, M., Pérez-Arellano, J. L., López-Gatius, F., Pascual-Velasco, F., Cilla, G., Rodriguez, N. F. and Anda, P. (2012) Molecular method for the characterization of Coxiella burnetii from clinical and environmental samples: variability of genotypes in Spain.BMC Microbiol. 2012;12:91 e22656068. [PMC free article] [PubMed]*
10. *R. Kuleye, Kuijt, M. A. Smit: H. I. J. Roest, H. E. Smith and A. Bossers (2017): Genome plasticity and polymorphisms in critical genes correlate with increased virulence of Dutch outbreak related Coxiella burnetii strains. Front. Microbiol.: 8: 1526.*
11. *McCaul T. F. and Williams, J. C. (1981): Development cycle of Coxiella burnetii: structure and free article), (Pub Med)*
12. *Omsland, Anders, Cockrell, Diane, C., Howe, Dale, Fischer, Elizabeth, R., Virtaneva, Kimmo, Sturdevant, Daniel, E.; Porcella, Stephen, F., Heinzen and Robert A. (2009), "Host cell-free growth of the Q fever bacterium Coxiella burnetii", PNAS;106(11):4430–4434, doi:10.1073/pnas.0812074106, PMC 2657411, PMID 19246385*
13. *Voth D. E and Heinzen (2007): Lounging in a lysosome: The intracellular lifestyle of Coxiella burnetii: Cell. Microbiol.;9(4):1829–1840, doi: 10.1111/j. 1462–5822.2007.0090 I.x. PMID 17381428 2009): Host cell free growth of Q fever bacterium Coxiella burnetii. Proc. Natl. Acad. Sci. USA.:106:4430 – 4434.[PMC free article] [PubMed]*

14. *Omsland Anders, Cockrell, Diane, C., Howe Dale, Fischer Elisabeth, R, Virtaneva, Kimmo, Surdevant, Daniel, E, Porcella, Stephen F. and Heizen Robert, A. (2009): Host cell free-growth of the Q fever bacterium Coxiella burnetii. PNAS;106(11):4430 – 4434, doi: 10.1073/pnas.0812074106. PMC 2657411@ PMID 19246385*

15. *Angelakis, E. and Raoult, D.: Q fever – Review. (2010), Vet. Microbiol.: 140:297 – 309. (Pub med)*

16. *Baca, O. G. and Paretsky D. (1983) Q fever and Coxiella burnetii: a model for host parasite interaction. Microbiol Rev.;47:127–149. [PMC free article] [PubMed]*

17. *Hackstadt T., Peacock M. G., Hitchcock P., J. and Cole R. L. (1985): Lipopolysaccharide variation in Coxiella burnetii: intrastrain heterogeneity in structure and antigenicity. Infec. Immun.;48:359–365. [PMC free article][PubMed]ch*

18. *Blondeau, J. M., Williams J. C. and Marrie T. J. (1990): The immune response to phase 1 and phase 2 Coxiella burnetii gens as measured by Western blotting. Ann NY Acad. Sci.;590:187 - 202 (Pub med).*

19. *Jado, I., Carranza-Rodriguez, C., Barandika, J. F., Toledo, A., Garcia-Amil, C., Serrano B., Bolos, M., Gil, H., Escudero, R., Garcia-Perez A. L., Olmeda, A. S., Astobiza, I., Lobo B., Rodriguez-Vargas, M., Perey-Arellano J. L., Lopez-Gatius, F., Pascular-Velasco, F., Cilla G., Rodriguez, N. F. and Anda, P.(2012): Molecular method for the characterization of Coxiella burnetii from clinical and environmental samples : variability of genotypes in Spain. MBC Microbiol: 12: 91 e 22656068 (PMC free article) (Pub med)*

20. *Van Der Hoek, W., Wielders, C. C. H., Schimmer, B., Wegdamblans, M. C. A., Meekenlenkamp, J., Zaaierrr, H. L. and Schneeberger, P. M. (2012): Detection of phase I IgG antibodies to Coxiella burnetii with ELISA as a screening test for blood donations. Eur. J. Clin. Microbiol. Infect. Dis.; 31:3207 – 3209 (Pub med).*

21. *Anja Scholzen, Guilhem Richard, Leonard Moised and Anja Garitsen (2019): Coxiella burnetii epitope specific T-cell responses in chronic Q fever patients. Infect. Immun. Doi 10.11.28/IAI 00213–19, 334633229 (1).*

22. *Hackstadt, T., Peacock, M. G., Hitchcock, P. J., and Cole, R.L. (1985): Lipopolysaccharide variation in Coxiella burnetii: inrastrain heterogeneity in structure and antigenicity. Infect. Immun.;48:359 – 365 (PMC free article) (Pub med)*

23. *Hove, D. and L. P. Malavia (2000): Coxiella burnetii exhibit morphological change and delays phagolysosomal fusion after internalization by J774 A1 cells. Infect. Immun. 68:3815 – 3822.*

24. *Mosavi, L. K., T. J. Cammet, O. C. Desrosiers and Z. Ypeng (2004): The ankyrin protein repeat as molecular architecture for protein recognition. Protein Sci.;13:1435 – 1448.*

25. *Lepidi, H., Hopikien, P., Liang, Z., Rault, and D. (2000): Cardiac valves in patient with Q fever endocarditis. Microbiological and histologic studies. J. Infect. Dis.;30:633 – 638. (Pub med).*

26. *D. Duron, K. Sidi-Boumydine, E. Rousset, S. Moutailer and E. Jourdain (2015): The importance of Ticks in Q fever transmission - What Has (and Has Not) Beeks demonstrated ? Trend in Parasitology, vol.;31:11:536 – 552.*

27. *Omsland, A., Cockrell, D. C., Howe, D, Fischer, E. R., Virtaneva, K., Sturdevant, D. E., Porcella and S. F., Heinzen, R. A. (2012): Host Epidemic genotype of Coxiella burnetii among goats, sheep and humans in the Nederland. Emerg. Infect. Dis.;8:887 – 889.*

28. *Madariaga, M. G., Rezai, K., Trenholme, G. M. and Weinstein, R. A. (2003): Q fever: a biological weapon in your backyard. Lancet. Infect. Dis. 2003;3:709–721. [PubMed]*
29. *Stocker, M. G. P. and Marmion, B. P. (1955): The spread of Q fever from animals to man. The natural history of a rickettsial disease. Bull WHO. 1955;13:781–806. [PMC free article] [PubMed]*
30. *Miceli, M., Veryser, A. K., Anderson, A. D., Hofinger, D., Lee, S. A., and Tancik, C. (2010): A case of person-to-person transmission of Q fever from an active duty serviceman to his spouse. Vector Borne Zoonotic Dis. 2010;10:539–541. [PubMed]*
31. *Editorial comment on Q fever transmitted by blood transfusion – United States. (2000). Cur. Dis. Weekly Rep.*
32. *Kruszewska, D. and Tylewska-Wierzbanowska, S. T.(1997): Isolation of Coxiella burnetii from bull semen. Res. Vet. Sci. 1997;62:299–300. [PubMed]. 1977;3:210.*
33. *Fournier, P. E., Marrie, and T. J., Raoult, D. (1998): Diagnosis of Q fever. J. Clin. Microbiol.;36:1823–1834.[PMC free article] [PubMed]*
34. *Syrucek, L., Sobelavsky, O., and Gutvirth, I. (1958): Isolation of Coxiella burnetii from human placentas. J. Hyg. Epidemiol. Microbiol. Immunol.;2:29–35. [PubMed]*
35. *Raoult, D., Levy, P. Y., Dupont, H. T., Chicheportiche, C., Tamalet, C., Gastaut, J. A., and Salducci, J. (1993): Q fever and HIV infection. AIDS. 1993;7:81–86. [PubMed]*
36. *Kampschreur, L. M., Dekker, S., Hagenaars, J. C. J. P., Lestrade, P. J., Renders N. H. M., De Jager-Leclercq, M. G. L., Hermans M. H. A., Groot, C. A. R., Groenwold, R. H. H., Hoepelman, A. L. M., Wever, P. C. and Oosterheert, J. J. (2012): Identification of risk factors for chronic Q fever, the Netherlands. Emerg. Infect. Dis.; 18:563–570. [PMC free article][PubMed]*
37. *Palmer, S. R. and Young, S. E. J. (1982): Q fever endocarditis in England and Wales, 1975 - 1981. Lancet. 1982;2:1448–1449.[PubMed]*
38. *Morguet, A. J., Jansen, A., Raoult, D. and Schneider, T. (2007): Late relapse of Q fever endocarditis. Clin. Res. Cardiol.;96:519–521. [PubMed]*
39. *Beslagic, E., Hamzic, S., Puvacic, S. and Cavaljuga-Hotic, S. (2003): Q fever serologic diagnostics with inhabitants of Sarajevo 2001 year. Med. Arh.;57:71–74. [PubMed]*
40. *Teunis, P. F. M., Schimmer, B., Notermans, D. W., Leenders, A. C. A. P., Wever, P. C., Kretzschmar, M. E. E., and Schneeberger, P.M. (2013): Time-course of antibody responses against Coxiella burnetii following acute Q fever. Epidemiol. Infect.;141:62–730. [PubMed]*
41. *Berri, M., Arricau-Bouvery, N. and Rodolakis, A. (2003): PCR-based detection of Coxiella burnetii from clinical samples. Meth. Mol. Biol.;216:153–161. [PubMed]*
42. *Blondeau, J. M., Williams, J. C., and Marrie, T. J. (1990) : The immune response to phase 1 and phase 2 Coxiella burnetii antigens as measured by Western blotting. Ann N. Y. and Acd. Sci.;590:187–202. [PubMed]*

43. *Kilic, S., Yilmaz, G. R., Komiya, T., Kurtoglu, Y. and Karakoc, E. A. (2008): Prevalence of Coxiella burnetii antibodies in blood donors in Ankara, Central Anatolia, Turkey. New Microbiol.;31:527–534. [PubMed]*

44. *Marrie, T. J. and Raoult D., (1992): Rickettsial infections of the Central nervous system. Semin. Neurol.:12:213–224 (Pub med).*

45. *Udaundo, P., Garcia-Delpech, S., Salom D., Garcia-Pous M. and Dias Liopis, M. (2011): Q fever a new ocular manifestation. Clin. Opthalmol., 5:1273 – 1275 (PMC free article) (Pub med)*

46. *Yeaman, M. R., Mitscher, L. A., and Baca, O. G. (1987): In vitro susceptibility of Coxiella burnetii for antibiotics, including several quinolones. Antimicrob. Agents Chemother.;31:1079–1084. [PMC free article] [PubMed]*

47. *Spyridaki, I., Psaroulaki, A., Vranakis, J., Tselentis, Y. and Gikas, A. (2009): Bacteriostatic and bactericidal activities of tigecycline against Coxiella burnetii and comparison with those of six other antibiotics. Antimicrob. Agents Chemother.;53:2690–2692. [PMC free article] [PubMed]*

48. *De Cremonony R., Rousset E., Touratier A., Audusseau G., Le Pape M. and David V. (2012): Assesment of vaccination in goat herds in Clinical Q fever situation. FEMS Immuno. Med. Microbiol.;64:104–106 [PubMed]*

49. *Robins, F. C. (1946): Q fever in the Mediterranean area: report on its occurrence in Allied Troops. Am. J. Hygiene. 1946;49:51–71.[PubMed]*

50. *Lecronier, M., Prendki, V., Gerin, M., et al. (2013): Q fever and Mediterranean spotted fever associated with hemophagocytic syndrome: case study and literature review. Int. J. Infect. Dis. 2013;17(8):e629–e633. doi:10.1016/j.ijid.2012.12.026PubMed*

51. *Georgiev, M., Afonso, A., Neubauer, G., Needham, H., Thiery, R., Rodolakis, A., Roest, H. J., Stark, K. D., Stegeman J. A., Vellema, P., et al. (2018): Q fever in humans and farm animals in four European countries, 1982–epizootic features of a Q fever outbreak in the border region between Serbia and Montenegro, J. Infect. Dev. Countr. (JICD);12(5):290–296, doi10.3855/ JICD 9918.*

52. *Zoran Debeljak, Snezana Melic, Marija Baralic, Aleksandra Andric, Aleksandar Tomic, Dejan Vidanovic, Milanko Sekler and Kazimir Matovic (2018): Clinical, epidemiological and epizootic features of a Q fever outbreak in the Montenegro.;12(5):290–296 doi.10.3855/JIDC9918. J. Infect. Dev. Countr. (JIDC)*

53. *Dejan Lausevic (2000): Examination of the presence of Q fever in sheep in the epizootic 'area of Montenegro. Master's thesis, Faculty of Veterinary Medicine in Belgrade, Serbia, 2000.*

54. *Institute of public health of Montenegro (2015) Health statistical yearbook 2014 of the Republic of Montenegro. Available http://www.ijzcg.me/2016/12/statisticki-godisnjakza-2014-godinu. Accessed 03 March 2017. [Report in Montenegrin]. 14. Institute of Public Health of Montenegro (2017)*

55. *Andric, B., Lako, B., Samardzija, N. M., Vukadinov, J., Jovanovic, J. and Canak, G. (2003): Rickets disease, characteristics and significance in modern infectious pathology. Acta Infectologica Yugoslavica 2003: 23*

56. *Andric, B., Lausevic, D., Lako, B., et al. (2005): Q fever in human and veterinary pathology in Montenegro. Veterinaria;54(3–4):191–198, Sarajevo, Yugoslavia.*

57. *Mahamat, A., Edouard, S., Demar, M., et al. (2013): Unique clone of Coxiella burnetii causing severe Q fever, French Guiana. Emerg. Infect. Dis.;19(7):1102–1104. doi:10.3201/eid1907.130044PubMed.*

58. *Morguet, A. J., Jansen, A., Raoult, D. and Schneider, T. (2007): Late relapse of Q fever endocarditis. Clin. Res. Cardiol.;96:519–521. [PubMed]*

59. *Lepidi, H., Houpikian, P., Liang, Z. and Raoult, D. (2003) : Cardiac valves in patients with Q fever endocarditis: microbiological, molecular, and histologic studies. J. Infect. Dis.187(7):1097–1106.doi:10.1086/368219. [PubMed]*

60. *60. Botelho-Nevers E., Fournier, P.-E., Richet H., et al. (2007): Coxiella burnetii infection of aortic aneurysms or vascular grafts: report of 30 new cases and evaluation of outcome. Eur. J. Clin. Microbiol. Infect. Dis.;26(9):635–640. doi:10.1007/s10096-007-0357-6. [PubMed]*

61. *Karhof, S., van Roeden, S. E., Oosterheert J. J., et al. (2018): Primary and secondary arterial fistulas during chronic Q fever [published online April 20, 2018]. J. Vass. Surg. doi:10.1016/j.jvs.2018.01.044 [PubMed]*

62. *TBWA, M., Hertogh, D. G., Neuville, B., Roskams, T., Nevens, F. and Steenbergen, V. W. (2001): Hepatic fibrin ring granulomas in granulomatous hepatitis: report of four cases and review of literature. Acta Clin. Belg.;56:341–348.[PubMed]*

63. *Mahamat, A., Edouard, S., Demar, M., et al. (2013) : Unique clone of Coxiella burnetii causing severe Q fever, French Guiana. Emerg. Infect. Dis.; 19(7):1102–1104. doi:10.3201/eid1907.130044. [PubMed]*

64. *Epelboin, L., Chesnais, C., Boullé, C., et al. (2012) : Q fever pneumonia in French Guiana: prevalence, risk factors and prognostic score. Clin. Infect. Dis.;55(1):67–74.doi:10.1093/cid/cis288 [PubMed]*

65. *de Lange, M. M. A., Hukkelhoven, C. W. P.M, Munster, J. M., Schneeberger P. M. and van der Hoek W. (2015) : Nationwide registry-based ecological analysis of Q fever incidence and pregnancy outcome during an outbreak in the Netherlands. BMJ Open.;5(4):e006821. doi:10.1136/bmjopen-2014-006821 [PubMed]*

66. *Million, M., Roblot, F., Carles, D., et al. (2014): Reevaluation of the risk of fetal death and malformation after Q fever. Clin. Infect. Dis.;59(2):256–260. doi:10.1093/cid/ciu259 [PubMed]*

67. *Million, M., Thuny, F., Bardin, N., et al. (2016) : Antiphospholipid antibody syndrome with valvular vegetations in acute Q fever. Clin. Infect. Dis. 2016;62(5):537–544. [PubMed]*

68. *Bernit, E., Pouget, J., Janbon, F., et al. (200): Neurological involvement in acute Q fever: a report of 29 cases and review of the literature. Arch. Intern. Med.;162(6):693–700. doi:10.1001/archinte.162.6.693. [PubMed]*

69. *Kofteridis, D. P., Mazokopakis, E. E., Tselentis, Y. and Gikas, A. (2004): Neurological complications of acute Q fever infection. Eur. J. Epidemiol.;19(11):1051–1054. doi:10.1007/s10654-004-0108-2 [PubMed]*

70. *Sawaishi, Y., Takahashi, I., Hirayama, Y., et al. (199): Acute cerebellitis caused by Coxiella burnetii. Ann. Neurol. 1999;45(1):124–127. doi:10.1002/1531-8249(199901)45:1<124::AID-ART19>3.0.CO;2-B [PubMed]*

71. *Vogiatzis, I., Dimoglou, G. and Sachpekidis, V. (2008) : Q fever myocarditis. Hippokratia. 2008;12(1):46–49. [PubMed]*

72. *Deller, M. and Streiff, E. (1956): Retrobulbar neuritis and Q fever [in French]. Bull. Mem. Soc. Fr. Ophtalmol. :309–314.*

73. *Udaondo, P., Garcia-Delpech, S., Salom, D., Garcia-Pous M. and Diaz-Llopis, and M. (2011) : Q fever: a new ocular manifestation. Clin. Ophthalmol.;5:1273–1275.doi:10.2147/OPTH.S18771 [PubMed]*

74. *Ergas, D., Abdul-Hai, A., Sthoeger, Z. M. (2008) : Acalculous cholecystitis: an unusual presentation of acute Q fever masquerading as infectious endocarditis. Am. J. Med. Sci.;336(4):356–357. doi:10.1097/MAJ.0b013e31815bf9f9 [PubMed]*