Emerging Aspects of *Coxiella burnetii* and Molecular Identification along with Treatment Approaches

Arslan Habib\(^1\), Zeeshan Ashraf\(^2\), Muhammad Nabeel\(^3\), Dominic Kwesi Quainoo\(^4\), Umutumwa Eric Principe\(^5\), Naeem Ullah Khalil\(^6\), Muhammad Rizwan Ullah\(^7\) and Bilal Khalid\(^8\)

\(^1\)Laboratory of Molecular Immunology, School of Life Sciences, Fudan University, Shanghai, China.
\(^2\)Department of Fisheries & Aquaculture, University of Veterinary and Animal Sciences, Lahore, Pakistan.
\(^3\)Knowledge Unit of Science and Technology, Faculty of Sciences, University of Management and Technology, Pakistan.
\(^4\)Department of Biotechnology, Faculty of Biosciences, University for Development Studies, Ghana.
\(^5\)School of Biotechnology, East China University of Science & Technology, Shanghai, China.
\(^6\)Centre of Biotechnology and Microbiology, Faculty of Life Sciences, University of Peshawar, Pakistan.
\(^7\)Department of Zoology, University of Lakki Marwat, Pakistan.
\(^8\)Institute of Zoology, University of the Punjab, Lahore, Pakistan.

Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJRID/2021/v7i230214

Editor(s):
(1) Dr. Hetal Pandya, SBKS Medical Institute & Research Center, India.

Reviewers:
(1) Samuel Badu Nyarko, University of Cape Coast, Ghana.
(2) Davoud Balarak, Zahedan University of Medical Sciences, Iran.

Complete Peer review History: https://www.sdiarticle4.com/review-history/70278

Received 27 April 2021
Accepted 02 July 2021
Published 13 July 2021

ABSTRACT

Q fever is an infectious disease caused by *Coxiella burnetii* which is an obligatory intracellular parasite. Globally Q fever is a widespread zoonosis. It is characterized by headaches, sudden fever and atypical pneumonia. In the rural environment, the main reservoir includes goats, sheep, cows, dogs, cats and rabbits. The main reservoir of this bacteria is considered domestic animals. They produce in a large number in amniotic fluid and placenta during childbirth. The main route of
infection is inhalation. Q fever can cause both acute and chronic infection, mostly asymptomatic in humans and animals. Inactivated whole-cell bacteria vaccination strategy has been performed which provides effective outcomes in humans and animals but many side effects have been observed. The recombinant vaccine has been developed and provides many effective results in experimental conditions. One of the major challenges is the lack of accurate diagnosis facilities if it becomes possible, the prognosis of disease development can be reduced. Direct detection of bacteria is the accurate test for the diagnosis. Different procedures are involved in this method such as immunodetection, PCR amplification and shell vial cell culture. Due to the severe infectivity of C. burnetii all these procedures require a biosafety level 3 lab and qualified staff. Q fever is a challenging disease for scientists to reduce its burden globally. The review discloses the Coxiella burnetii genome, the clinical manifestation of Q fever as well as emerging issues, diagnosis, treatment, prevention and future directions.

Keywords: Coxiella burnetii; diagnosis; Q fever; prevention; treatment.

1. INTRODUCTION

Globally Q fever (Latin: debris Q, coxiellosis) infected different animal species along with humans. The causative agent of Q fever is a unique phylogenetic and taxonomical bacterium (C. burnetii), which produces spores intracellularly that are impervious to different environmental factors. In 1935 the disease was first time recognized and in 1937 Derrick explained this disease during his research among abattoir workers in Australia with unknown disease outbreaks [1]. Q fever occurs globally, most cases of such disease victims are reported in Australia and southern France. Different kinds of hosts get infected with C. burnetii which includes pets, ruminants, humans and a few ticks, reptiles and birds. The discharge of bacterium is found in the birth products, urine, feces and milk. Such products consist of many bacteria that become aerosolized after being deceased. A few organisms are responsible for the prevalence of C. burnetii infections. C. burnetii can persist viable and toxic for months due to its spore-like-life cycle. Transmission of infection occurred through inhalation or skin exposure and direct contact with a ruminant is not a risk factor for infection. Transmission of infection from human to human is rare except for some exceptional cases such as infected blood transfusion and placenta of infected female [2]. Sexual transmission is also a risk of infection. In humans, asymptomatic infection of C. burnetii is common or some influenza-like illness or atypical pneumonia are observed. C. burnetii infection in few cases (<5%) becomes chronic with noxious outcomes especially in patients with pre-existing valvular heart disease [3]. C. burnetii is identified as a latent agent of bioterrorism because of its major infectious nature and acquiring inhalational mechanism of transmission [4]. The bacterium is highly viable and toxic because of its virulence to cause infection. Different routes are possible risk factors of infection transmission including exposure to contaminated milk, wool, meat and inhalation of contaminated dust and importantly birth products. Ticks are also involved in the transmission of pathogenic agents to other animals [5-8].

1.1 C. burnetii Entry and Survival in the Host Cells

Different kinds of cells are infected by the C. burnetii which includes macrophages, monocytes, in vivo, in vitro and diversity of transformed cells such as VERO cells, L929, or HEla [9]. The possible factor of the virulence of C. burnetii is its magnitude to occupy and thereafter to mature within such eukaryotic cells which permits its multiplication in different niches of the host [10]. The acidified environment is the suitable environment for its survival and multiplication which they complete in the phagolysosome. Different kinds of reviews explained the C. burnetii bacterial factors used for the intracellular survival and biphasic developmental cycle in the host cell [11,12]. The small and large appearance possess different genes that allow the bacterium to survive in the particular severe niche of each form. For instance, during the stationary period in major bacteria RpoS (sigma S) is a sigma subunit that negotiates promoter-specific transcriptional induction by RNA polymerase to genes. RpoS causes different physiological and morphological modifications along with multi-stress resistance. LCV exhibits a large number of RpoS while SCV does not possess notable RpoS [13], expressed that LCV and SCV multiplications adaptations may not be the functional parallel types of logarithmic and stationary period bacteria as...
suggested initially [14]. During the surviving pressure in metabolically active LCV, this sigma factor may synchronize genes [15]. Therefore, the small types are proficient to detain phagolysosomal fusion, possibly to promote the adaptation from SCV to LCV that present at pH 5.5 in the endosome [16]. Larger duplicative vacuoles possessing Coxiella are obtained from fusion with late endosomal-lysosomal organelles by the use of cellular markers such as rab7, LAMP-1 and EEA.1 [17,18]. The secretion system should be associated with the development of phagosome containing Coxiella suggested by the sequence homologies between the type IV secretion system proteins of Coxiella and Legionella [19]. In fact, during in vivo host cell infection, Coxiella encodes functional constituents of the type IV secretion system [20]. While the C. burnetii secretion system is mechanistically associated with the Legionella Dot/Icm system even, C. burnetii IcmQ protein and the Legionella IcmR protein do not interrelate with each other [20,21]. For producing exclusive vacuole that assists C. burnetii replication this secretion mechanism could play an important role. The assimilation of virulent C. burnetii depends on phase I LPS on TLR4 and αβ3 integrin [22]. Early occasions of C. burnetii infection controls by TLR4 which includes cytokine production, granuloma formation and macrophage phagocytosis [23]. This receptor is not limited to humans but also associated with insects [24,25]. Therefore, C. burnetii has adapted a cell utilization method that permits it to enter an exclusive host range and has established a specific tactic to replicate in the adverse phagolysosome environment of the cells. In fact, from different hosts, C. burnetii has been isolated such as mammals, amoeba, birds and ticks which adapted them to survive in the vast environment for long-term periods and to increase its prevalence.

2. CLINICAL MANIFESTATION

During the acute infection Q fever mostly express subclinical or highly mild symptoms. Approximately 20 days (range 14 to 39 days) incubation period has been observed. There is no particular type of Q fever and the clinical symptoms of infection differentiate from patient to patient. The epidemiological circumstance is the most principle diagnostic indicator. Some of the major descriptions are as follows:

(i) The self-confined flu-like syndrome is the maximum prominent manifestation of q fever. In Spain, 21% of the occurrence of this shape of q fever has been illustrated which lasting for multiple weeks and less than 3 weeks. The most possible clinical signs encompass excessive-grade fever (104◦ for 40 •C), myalgias, unexpected onset, fatigue and headache. Growing age reasons an increase within the duration of fever.

(ii) One of the maximums regularly diagnosed styles of acute q fever is abnormal pneumonia. Most inflamed individuals expressed clinically asymptomatic or moderate, recognized via an ineffectual cough, fever and negligible auscultatory abnormalities, but a few individuals expressed acute breathing misery. Pleural effusion also can be determined. Detection on the chest radiograph is negligent. Findings on the chest radiograph are nonspecific. Marrie et al. [26] expressed that 3.7% of all infected individuals with community attained pneumonia confessed to a tertiary-care teaching health center in nova scotia over five years have been due to c. Burnetii, which is correlated to the results of Lieberman et al. Israel (5.8%) [27].

(iii) Hepatitis (inflammation of the liver) is the main type of acute q fever and it reveals particularly granulomatous hepatitis. Variable length of clinical symptoms turned into discovered from 10 to ninety days. The mortality rate relies upon attention ranging from 0.5 to 1.5% [28]. 3 fundamental forms of hepatitis may be involved together with clinically asymptomatic hepatitis, an infectious shape of hepatitis with hepatomegaly however very so often jaundice and unknown foundation persevered fever with particular granulomas on liver biopsy [29].

(iv) Many different exclusive varieties of acute q fever scientific manifestations are feasible together with pericarditis, myocarditis, headache, maculopapular or purpuric exanthema in 10% of patients [28]. Aseptic meningitis and encephalitis with q fever which accounts for 0.2-1.3% of infected individuals are occasionally attended with the aid of seizures and coma [30]. Hemolytic anemia, gastroenteritis, erythema nodosum, inappropriate secretion of antidiuretic hormone and splenic rupture are unfamiliar manifestations of acute q fever.
2.1 Emerging and Re-Emerging Aspect

Different published research findings on the Q fever outbreak and reflective studies of isolated cases or unfamiliar clinical expressions have been flourishing since 1999 (Table 1). Similarly, this rise about the outbreak may influence the awareness status among the community rather than its emergence. Seroprevalence of animal Q-fever obtained in the main recent worldwide surveys mentioned in Table 2. Distribution of confirmed Q fever cases and rates per 100 000 population by country and year, EU/EEA, 2014–2018 mentioned in Table 3 and Fig 1. Clinical signs in humans may fluctuate according to the region as well as the age and gender of the infected individuals. Most commonly asymptomatic infection was observed among the children and non-pregnant women. Among the older patient’s atypical pneumonia is the major possible Q fever manifestation [31] and was observed in the different countries of a reported outbreak among the infected individuals such as (Nova Scotia Canada [32], Greek Island of Crete [33], Italy [34], Japan [35], Switzerland [36] and the United Kingdom). In some other countries like France, California and Australia [37], prolonged fever and hepatitis are the most familiar manifestations. Atypical pneumonia was observed in Spain and specifically in Catalonia [38] and the Basque country [39], while hepatitis and prolonged fever-like symptoms were observed in Andalusia [40]. The description of such variability is still under observation and could be due to the genetic variations among the strains. *C. burnetii* strains isolated from ticks are more virulent as compared to those isolated from bovine milk experimented in a guinea pig model [41]. Furthermore, it would be provocative to understand if virulence factors of the bacteria could be the cause of intensity of the disease in some caprine flocks where pregnant females aborted nearly 30% while were asymptomatic but eliminate the bacteria for several months. Currently, still, there are missing genomic markers and epidemiological antigenic or under the developmental stage. However, recent research has utilized different approaches to study the prevalence of Q fever [42].

2.2 Coxiella burnetii Genome

The genome of *C. burnetii* Nine Mile phase I consists of a 1.9 Mb chromosome and plasmid QpH1 (37,393 bp) [43]. In the National Centre for Biotechnology Information (NCBI) database, there are 6 available genomes of *C. burnetii*, among which 4 are fully sequenced and 2 are still being analyzed (Table 4). All strains of *C. burnetii* possess 1 autonomous plasmid with a size ranging from 37–55 kb, and in some cases, plasmid sequences can be integrated with bacterial chromosomes (Table 5) [44]. High homology among plasmid sequences has led to the hypothesis that they are essential for the survival of the pathogen [45]. This hypothesis was confirmed with the PCR-RFLP (Polymerase Chain Reaction – Restriction Fragment Length Polymorphism) method, and as a result, 6 different genomic groups (genomic groups I-VI) have been characterized [44]. To ensure that the PCR-RFLP is a sufficient method, MLVA (Multilocus Variable Number Tandem Repeats Analysis) analysis [46] and the multispacer sequence typing (MST) method [47] were also performed. The results suggest that all of the above phylogenetic methods can be successfully used in the molecular analysis of *C. burnetii*. A novel approach in molecular testing was an application of the microarray method in the molecular characterization of isolated strains. This method revealed 2 new genomic groups of *C. burnetii* (VII and VIII) [48]. The molecular characteristics of *C. burnetii* strains also included sequencing and the PCR-RFLP of specific genes: icd (isocitrate dehydrogenase) [49], com1 (outer membrane protein) and mucZ (mucoid protein) [50].

2.3 Antibiotic Susceptibility and the Role of pH

For years, because of the obligate intracellular behavior of *C. burnetii* the antibiotic susceptibility trying out (AST) was arduous. Distinctive strategies had been used for the assessment of antibiotic activity as firstly observed in animal fashions than in embryonated egg models and the long run-in cell culture systems. At some stage in the early research inoculation in guinea pigs turned into implemented for susceptibility evaluation of streptomycin [51]. The potential of the examined antibiotic improved the survival duration in eggs infected with *C. Burnetii* was located throughout the embryonated-egg approach. To evaluate the ability of aureomycin, streptomycin, oxytetracycline and chloramphenicol against *C. Burnetii* this technique changed into carried out inside the older researches [52]. After that cell culture system was then performed and persist the reference technique for *C. burnetii* AST. In 1987, *C. burnetii* infected L929 fibroblast cells have been used by Yeaman et al. and differentiated the ratio of infected cells in antibiotic-treated cultures to that during drug-free controls [53].
Table. 1. Main outbreaks of Q fever described since 1999

| Country   | Outbreak     | Source            | No. of cases | Diagnosis | Ref. |
|-----------|--------------|-------------------|--------------|-----------|------|
| Italy     | 1987-1998    | Ovine             | 235          | ?         | [118]|
| France    | 1990-1995    | Ovine             | 289          | IF        | [119]|
| Kenya     | ?            | Goat              | 4            | IF        | [120]|
| Germany   | 1999         | Ovine, dung       | ?            | ?         | [121]|
| Netherland| ?            | Vacation in France| 4            | Serology  | [122]|
| Newfoundland| 1999     | Goat              | 60           | IF        | [123]|
| French Guiana| 1996-2000 | Wild reservoir    | 132          | IF        | [124]|
| Australia | 1998         | Ovine             | 33           | PCR       | [125]|
| Israel    | 1999         | ?                 | 16           | ELISA     | [126]|
| Japan     | ?            | Travel in Australia| 3           | IF & PCR  | [127]|
| France    | 1996         | Ovine             | 29           | IFI       | [128]|
| Japan     | ?            | Dogs & Cats       | 2            | IF & PCR  | [129]|
| Bosnia    | 1997         | Ovine             | 26           | Serology  | [130]|
| Australia | 2000         | ?                 | 16           | CFTf      | [131]|
| France    | 2000         | Ovine manure      | 5            | IF        | [132]|
| France    | 2000         | Goat manure       | 10           | IF        | [132]|
| France    | 2002         | Ovine             | 88           | IF        | [132]|
| Italy     | 2003         | Ovine             | 133          | IF        | [133]|

With the help of particular antibodies and the immunofluorescence assay, the more appropriate shell vial assay was developed for the detection of intracellular *C. burnetii* [54]. Brennan and Samuel in 2003 by using quantitative PCR (qPCR) developed a modified shell vial assay for the identification of *C. burnetii* intracellular computation. This approach was more sensitive and observable as compared to the indirect immunofluorescence assay (IFA) [55]. One more experiment confirmed its magnificent reliability for MIC confirmation [56]. In recent years, among the different eukaryotic cell lines, both the IFA and qPCR experiments targeting *com1* or *apoB* have been used for *C. burnetii* AST [57]. Currently, with the usage of particular immunofluorescent probes and flow cytometry, a new method developed. This approach with its particular morphological characteristics permits a very susceptible counting of *C. burnetii* cells [58].

### 2.4 Main Susceptibility Features

Many studies reported that doxycycline is the most potent drug against *C. burnetii* with MICs of 2mg/liter [58]. Although, doxycycline resistant strains have been reported and express a...
### Table 2. Seroprevalence of animal Q-fever obtained in the main recent worldwide surveys

| Country | Year | No. of Herds | No. of Animals | Seroprevalence of animals (% of herds) | Diagnosis | Ref. |
|---------|------|--------------|----------------|----------------------------------------|-----------|-----|
| Goat    |      |              |                |                                        |           |     |
| Germany | 1999 | 1            | 100            | 57                                     | ELISA     | [111]|
| Chad    | 2000 | 28           | 142            | 11                                     | ELISA     | [112]|
| Italy   | 2000 | 104          | 2155           | 47                                     | ELISA     | [113]|
| Sheep   |      |              |                |                                        |           |     |
| Germany | 1999 | 1            | 100            | 57                                     | ELISA     | [111]|
| Chad    | 2000 | 28           | 142            | 43                                     | ELISA     | [112]|
| Italy   | 2000 | 675          | 7194           | 38                                     | ELISA     | [113]|
| Cattle  |      |              |                |                                        |           |     |
| Italy   | 1998 | 21           | 544            | 13                                     | IF        | [114]|
| Germany | 1998 | ND           | 21 191         | 8                                      | ELISA     | [111]|
| Turkey  | 1998 | 48           | 416            | 6                                      | IF        | [115]|
| Chad    | 2000 | 19           | 195            | 37                                     | ELISA     | [112]|
| Other   |      |              |                |                                        |           |     |
| Indonesia | 1999 | 2         | 327 rats       | 0                                      | IF        | [116]|
| Chad    | 2000 | 14           | 142 camels     | 100                                    | ELISA     | [112]|
| Korea   | 2000 | ND           | 116 pet cats   | 9                                      | IF        | [117]|
| Japan   | 2003 | ND           | 316 pet cats   | 14                                     | IF        | [117]|

### Table 3. Distribution of confirmed Q fever cases and rates per 100 000 population by country and year, EU/EEA, 2014–2018

| Country     | 2014 Number | 2014 Rate | 2015 Number | 2015 Rate | 2016 Number | 2016 Rate | 2017 Number | 2017 Rate | 2018 Number | 2018 Rate | Reported cases | Rate | ASR | Confirmed cases |
|-------------|-------------|-----------|-------------|-----------|-------------|-----------|-------------|-----------|-------------|-----------|----------------|------|-----|-----------------|
| Malta       | 0           | 0.0       | 0           | 0.0       | 0           | 0.0       | 2           | 0.4       | 2           | 0.4       | 0.4            | 2    |     |                  |
| Norway      | 1           | 0.0       | 1           | 0.0       | 2           | 0.0       | 4           | 0.1       | 5           | 0.1       | 0.1            | 5    |     |                  |
| Iceland     | 0           | 0.0       | 0           | 0.0       | 0           | 0.0       | 0           | 0.0       | 0           | 0.0       | 0.0            | 0    |     |                  |
| Czech Republic | 0           | 0.0       | 1           | 0.0       | 2           | 0.0       | 0           | 0.0       | 1           | 0.0       | 0.0            | 1    |     |                  |
| Liechtenstein |          |           |             |           |             |           |             |           |             |           |                |      |     |                  |
| Slovenia    | 3           | 0.1       | 1           | 0.0       | 1           | 0.0       | 3           | 0.1       | 1           | 0.0       | 0.0            | 1    |     |                  |
| Bulgaria    | 15          | 0.2       | 15          | 0.2       | 17          | 0.2       | 28          | 0.4       | 47          | 0.6       | 0.6            | 45   |     |                  |
| Spain       | 77          | -         | 97          | -         | 330         | 0.7       | 379         | 0.8       | 418         | 0.7       | 0.7            | 313  |     |                  |
| Austria     |             |           |             |           |             |           |             |           |             |           |                |      |     |                  |
| Finland     | 0           | 0.0       | 3           | 0.1       | 2           | 0.0       | 4           | 0.1       | 2           | 0.0       | 0.0            | 2    |     |                  |
| United Kingdom | 60         | 0.1       | 21          | 0.0       | 34          | 0.1       | 21          | 0.0       | 19          | 0.0       | 0.0            | 19   |     |                  |
| Country     | 2014 Number | Rate | 2015 Number | Rate | 2016 Number | Rate | 2017 Number | Rate | 2018 Number | Rate | Reported cases | Rate | ASR | Confirmed cases |
|-------------|-------------|------|-------------|------|-------------|------|-------------|------|-------------|------|----------------|------|-----|----------------|
| Croatia     | 21          | 0.5  | 14          | 0.3  | 8           | 0.2  | 23          | 0.6  | 16          | 0.3  | 0.3           | 0.3  | 11 |                 |
| Germany     | 238         | 0.3  | 310         | 0.4  | 270         | 0.3  | 107         | 0.1  | 93          | 0.1  | 0.1           | 0.1  | 90 |                 |
| Romania     | 21          | 0.1  | 3           | 0.0  | 32          | 0.2  | 46          | 0.2  | 22          | 0.1  | 0.1           | 0.1  | 22 |                 |
| Denmark     | -           | -    | 0           | 0.0  | 0           | 0.0  | 0           | 0.0  | 0           | 0.0  | 0.0           | 0.0  | 0  |                 |
| Italy       | -           | -    | -           | -    | 3           | 0.0  | 7           | 0.0  | 1           | 0.0  | 0.0           | 0.0  | 1  |                 |
| Sweden      | 2           | 0.0  | 4           | 0.0  | 3           | 0.0  | 1           | 0.0  | 7           | 0.1  | 0.1           | 0.1  | 7  |                 |
| Belgium     | 3           | 0.0  | 8           | 0.1  | 16          | 0.1  | 7           | 0.1  | 18          | 0.1  | 0.1           | 0.1  | 6  |                 |
| France      | 209         | 0.3  | 250         | 0.4  | 251         | 0.4  | 194         | 0.3  | 172         | 0.3  | 0.3           | 0.3  | 172 |                 |
| Poland      | 1           | 0.0  | 0           | 0.0  | 0           | 0.0  | 0           | 0.0  | 0           | 0.0  | 0.0           | 0.0  | 0  |                 |
| Cyprus      | 1           | 0.1  | 4           | 0.5  | 2           | 0.2  | 3           | 0.4  | 1           | 0.0  | 0.0           | 0.0  | 0  |                 |
| Ireland     | 0           | 0.0  | 4           | 0.1  | 6           | 0.1  | 2           | 0.0  | 0           | 0.0  | 0.0           | 0.0  | 0  |                 |
| Slovakia    | 1           | 0.0  | 0           | 0.0  | 0           | 0.0  | 0           | 0.0  | 0           | 0.0  | 0.0           | 0.0  | 0  |                 |
| Estonia     | 0           | 0.0  | 0           | 0.0  | 0           | 0.0  | 0           | 0.0  | 0           | 0.0  | 0.0           | 0.0  | 0  |                 |
| Portugal    | 25          | 0.2  | 20          | 0.2  | 17          | 0.2  | 48          | 0.5  | 36          | 0.3  | 0.3           | 0.3  | 36 |                 |
| Greece      | 15          | 0.1  | 10          | 0.1  | 9           | 0.1  | 4           | 0.0  | 13          | 0.1  | 0.1           | 0.1  | 13 |                 |
| Netherlands | 26          | 0.2  | 20          | 0.1  | 14          | 0.1  | 22          | 0.1  | 18          | 0.1  | 0.1           | 0.1  | 18 |                 |
| Hungary     | 59          | 0.6  | 35          | 0.4  | 39          | 0.4  | 29          | 0.3  | 28          | 0.3  | 0.3           | 0.3  | 28 |                 |
| Lithuania   | 0           | 0.0  | 0           | 0.0  | 0           | 0.0  | 0           | 0.0  | 0           | 0.0  | 0.0           | 0.0  | 0  |                 |
| Latvia      | 3           | 0.1  | 1           | 0.1  | 0           | 0.0  | 0           | 0.0  | 0           | 0.0  | 0.0           | 0.0  | 0  |                 |
| Luxembourg  | 0           | 0.0  | 1           | 0.2  | 0           | 0.0  | 0           | 0.0  | 0           | 0.0  | 0.0           | 0.0  | 0  |                 |
| EU/EEA      | 781         | 0.2  | 823         | 0.2  | 1058        | 0.2  | 932         | 0.2  | 922         | 0.2  | 0.2           | 0.2  | 794 |                 |

Table 4. Comparison of *C. burnetii* genomes

| Properties                  | Dugway | G    | K    | Nine Mile |
|-----------------------------|--------|------|------|-----------|
| Size of chromosome (bp)     | 2,158,758 | 2,008,870 | 2,063,100 | 1,995,281 |
| Coding regions (%)          | 90.7   | 28.7 | 90.3 | 90.7      |
| GC content (%)              | 42.4   | 42.6 | 42.7 | 42.7      |
| Total ORFs                  | 2,265  | 2,300 | 2,325 | 2,227     |
| Known function              | 1,391  | 1,403 | 1,441 | 1,348     |
| Unknown function            | 874    | 897  | 884  | 879       |
| Pseudogenes                 | 265    | 484  | 476  | 413       |
| Transposases                | 32     | 40   | 59   | 31        |

*C. burnetii* strain Dugway, *C. burnetii* strain G, *C. burnetii* strain K, *C. burnetii* Nine Mile phase I [134]
harassing condition. Firstly, a patient died from C. burnetii endocarditis recognized with a resistant strain. By using the shell vial assay and qPCR the doxycycline minimum inhibitory concentration (MIC) of 8 mg/liter was reported [59]. Meanwhile, Rolain et al. during the same study observed an association between the percentage of serum concentration to MIC for doxycycline and the tendency of reduced anti-C. burnetii antibody titers in patients infected with C. burnetii endocarditis. For 16 C. burnetii strains isolated from cardiac valves extract from endocarditis patients, a percentage of serum concentration to MIC of 1 associated with a prompt reduction in particular antibody titers. The percentage between 0.5 and 1 was correlated with a steady minimization in antibody titers [61]. The entire genome of the C. burnetii strain infecting that patient (Cb109) was observed, but no particular sequence could be associated with doxycycline resistance [60]. Similarly, two other doxycycline resistant strains have been observed such as human isolate associated with acute Q fever and another from goat isolate [61].

During the early studies, for the elimination of C. burnetii from L929 cells fluoroquinolones were reported to be one of the most potential agents [62]. For that purpose, in 1989 to treat resistant isolates of C. burnetii infection it was suggested to incorporate doxycycline with fluoroquinolone drugs [63]. Fluoroquinolones due to their effective cerebrospinal fluid insertion are also suggested for the treatment of acute meningitis caused by C. burnetii [64]. Spyridaki et al. and Musso et al., have determined in vitro pefloxac in or ciprofloxacin-resistant strains of C. Burnetii with MICs as much as sixty-four mg/liter [65]. For the PCR- restriction fragment length polymorphism (PCR-RFLP) identifications of those resistant isolates, these authors detected factor mutations in the gyrA gene [66]. However, thus far, clinical strains of C. burnetii persist susceptible to moxifloxacin, levofloxacin and to a minimal with ciprofloxacin [67].

For the treatment of C. burnetii pneumonia, erythromycin was suggested as a sustainable approach. Meanwhile, Raoult et al. in 1991, observed resistance to this antibiotic in almost 6 out of 13 clinical isolates of C. burnetii [68] and in Cayenne, French Guiana such resistance was more recently recognized in 6 isolates [69]. Therefore, clarithromycin with MICs between 2 and 4 mg/liter was observed to be active [70]. A higher MICs up to 8 mg/liter has been reported with azithromycin [68]. Telithromycin with MICs between 0.5 and 2 mg/liter was observed active against C. burnetii for 13 clinical isolates [61]. But recently a resistant strain to this antibiotic was reported from French Guiana [58].

Thus far, no resistance to sulfamethoxazole-trimethoprim has been determined, thinking about that this antibiotic is powerful all through being pregnant. Anecdotal reported susceptibility to tigecycline and linezolid and cautioned them as alternative dealers [70]. Unsworth et al. recently described the susceptibility of C. Burnetii to antimicrobial peptides [71]. In vitro movement in opposition to C. burnetii changed into located with different forms of nonantibiotics. During in vitro interest, it changed into located that lovastatin and pentamidine can retard the C. burnetii growth [72]. For minimizing the dimensions of C. burnetii intracellular vacuoles omeprazole is taken into consideration as effective [73].

2.4.1 Role of pH in persistent infection

For decades, the antibiotic treatment of steady C. burnetii infection is difficult because no antibiotic has uncovered a bactericidal impact. In 1990, antibiotic activity inhibited the multiplication of C. Burnetii within the phagolysosome- like vacuole for the duration of the acidic environment. At some stage in the in vitro persistent cell infection

| Properties          | Plasmid      |
|---------------------|--------------|
|                     | QpDG         | QpRS         | QpH1         |
| Size of plasmid (bp)| 54,179       | 39,280       | 37,393       |
| Coding regions (%)  | 84.9         | 79.6         | 81           |
| GC content (%)      | 39.8         | 39.7         | 39.3         |
| Total ORFs          | 66           | 48           | 50           |
| Known function      | 26           | 20           | 19           |
| Unknown function    | 40           | 28           | 32           |
| Pseudogenes         | 13 (7)       | 10 (6)       | 15 (10)      |
| Transposases        | 1            | 0            | 0            |

Table 5. Comparison of C. burnetii plasmids [134]
model, it became determined that acidification of the *C. burnetii* multiplication vacuole remained regular over time for 3 strains [74]. For that goal, Raoult et al. located a reconditioning of the bactericidal action of doxycycline after homogenization of alkalinizing retailers with doxycycline [75]. Chloroquine and amantadine had been used among alkalinizing marketers and healing bactericidal impact changed into accelerated with doxycycline. These results had been eventually validated clinically via differentiating the outcome of patients with *C. burnetii* endocarditis medicated with the association of either doxycycline at the side of fluoroquinolone or doxycycline with hydroxychloroquine. A shorter period of treatment and much less periodic relapses were found in many of the patients who benefited from later combinations [76].

### 3. LABORATORY DIAGNOSIS TOOLS

General routine laboratory way of life strategies is not suitable for the growth of *C. Burnetii*, which may be a motive for the diagnosis-specific indirect diagnostic tools are used. In addition, for trying out, *C. burnetii* infection serology is still the most familiar technique. Presently, qPCR is used for the detection of *C. burnetii* DNA from one-of-a-kind medical samples. Before seroconversion in patients with primary infection, it has the gain of detecting *C. burnetii*. A biosafety degree 3 (BSL3) laboratory is recommended for culturing identical scientific samples. Finally, after immunohistochemistry staining, a pathological exam of infected tissue samples is a provocative mechanism for prognosis when these samples are on hand. In recent years improvements in the sensitivity of the principle, diagnostic techniques have been the top target.

#### 3.1 Serological Methods

*Q* fever is difficult to diagnose due to an infectious disease. Clinical symptoms, serological outcomes and direct exposure with animals are the initial diagnosis approaches [77]. Serological methods such as IFA (Indirect immunofluorescence assay) and ELISA (Immunoenzymatic assay) are used for the detection of two forms of *C. burnetii*. The first form occupies smooth LPS detected the virulent phase also known as phase I. The second form occupies rough LPS also known as phase II [78]. Describing the normality of phase I and phase II antibodies permit determine the acute type from the chronic form of Q fever and is a forecast measuring the evolution of the acute type to the chronic type [79]. The necessity for clinical inspection, using, for example, 2-D echocardiography, and serological evaluation of recovering who expressed the acute form of the disease, for many years has been determined as vital in the phase of the probability to evolve the chronic form in these patients [80]. Currently, ECDC recognized this postulate as a standard method [81].

IFA and ELISA are commonly used to define the normality of antibodies against *C. burnetii*. Antigens of phase I and phase II are adapted in the IFA procedure, which is induced in murine epithelial placenta cells and fibroblasts of infected mice. This procedure permits acquiring large quantities of greatly specific antigens which can be used for the identification of IgG, IgM, and IgA antibodies [82]. Peter et al. [83] and Cowley et al. [84] expressed that ELISA and IFA can be used in routine serodiagnosis of Q fever, while ELISA is more strive and laborious and needed highly certified staff [82]. Antibody feedback to *C. burnetii* phase II antigen is prevalent and is greater than phase I antibody feedback during acute infection. An elevated phase I IgG normality was examined which is frequently excessive as compared to phase II IgG during the chronic infection.

Indirect immunofluorescence assay in association to *C. burnetii* antigen is the standard serological test for the detection of Q fever, which is carried out on paired serum samples to illustrate a significant (4-fold) increase in antibody normality. In many cases of Q fever observed that the first IgG IFA normality is usually low or negative and the second usually express a significant (4-fold) rise in IgG antibody levels. Antibodies concentration is usually considered positive for phase II above 1:64 for IgM and above 1:256 for IgG [85]. The serological diagnosis of humans for many years depends on 2 extremely specific tests: complement fixation test (CST) and microagglutination test in phases I and II, which carried out simultaneously [86]. Different kinds of other methods have also been practiced in the diagnosis of Q fever such as radioimmunological assays, complement fixation tests, microagglutination tests and western-blotting [82].

#### 3.2 Molecular Detection

For the identification of *C. Burnetii* in medical samples, various PCR-based total analyses have
been developed. The first perfect PCR systems targeted sequences of various varieties of plasmids [87], the 16s-23s RNA, the superoxide dismutase gene, the com1 gene or the IS1111 repetitive factors in human or animal samples [88]. Such different techniques ranged detection limits from 10 to 10² bacteria. Nested PCR strategies had been additionally encouraged however these systems lack accuracy [89]. Real-time PCR or quantitative PCR (qPCR) has the benefit of comparing the volume of bacteria in clinical samples and is a much less enormous approach than PCR. As a result, for the analysis, this method has emerged as the maximum generally used than others. The qPCR technique focused on IS1111 (a repetitive detail that is found in about 20 copies in the C. burnetii 9-mile genome) is the maximum sympathetic [90]. The modern-day qPCR technique inside the first 2 weeks of infection can stumble on the bacterium in the sera while the serological assay is not still high quality. Additionally, continual C. burnetii infection permits the identity of C. burnetii DNA in the blood of patients [91]. Schneeberger et al. within the Netherlands, found patients with signs of preliminary infection diagnosed with C. burnetii DNA in 10% of seronegative samples, verifying the effectiveness of this technique within the first weeks of infection [92]. One extra trial from the outbreak expressed sensitivity, specificity, PPV, and negative predictive value (NPV) of 92.2%, 98.9%, 99.2%, and 89.8%, respectively [92]. In that experiment, during the primary infection, a higher DNA load was correlated with advancement to persistent infection. Tilburg et al. used the DNA extraction technique among seven laboratories beyond the Netherlands and evaluated the interlaboratory compliance of IS1111 qPCR according to the method [93]. They observed that different associations of DNA extraction kits and qPCR analysis performed similar outcomes for Q fever detection. In Switzerland, for the diagnosis of C. burnetii in clinical samples a qPCR approach concentrated on the ompA gene has been carried out for 7 years [94]. The sensitivity became 50% for urine samples, 69% for blood samples and 88% for valvular samples. Another qPCR technique focused on IS30A repetitive factors expressed a decreased sensitivity than IS1111 qPCR [95].

Recently, multiplied the sensitivity of the qPCR test concentrated on the IS1111 gene by concentrating DNA extracted from medical samples by way of lyophilization [96]. The diagnostic extent of C. Burnetii DNA changed into the 100-fold decrease in lyophilized sera (1 bacterium/ml) compared to nonlyophilized sera (102 microorganism/ml). Almost seventy-three patients acquired this approach which was normally infected with C. burnetii and 10 samples from endocarditis patients in whom the IS1111 qPCR carried out under the normal conditions remained negative.

3.3 Culture

A huge variety of clinical samples can offer the possibility to isolate the C. Burnetii, along with old samples before cultivation which are stored at -80 C. The maximum normally used method is the shell vial technique [97]. On HEL cell monolayers in a shell vial, a pattern of one ml of the clinical specimen is inoculated. Centrifugation became achieved for shell vials (seven-hundred g at 20 C) for 1 h. Centrifugation permits more extension and perforation of C. burnetii inside cells. For 5-7 days inflamed cells are then incubated at 37°C in a 5% CO₂-enriched ecosystem. For the diagnosis of the bacterium internal cells, Gimenez or immunofluorescence staining turned into performed. Lockhart et al. making use of one-of-a-kind isolates, the Heinzingerling and Arrandale lines in affiliation to differentiate four different cell lines for the isolation of C. burnetii [98]. For the Arandale isolate, Vero cells expressed excessive sensitivity at the same time as, for the Heinzingerling strain, DH82 cells have been the most sensitive. For the culturing of C. burnetii L929 and XTC, cell lines have been much less appropriate.

Recently, in axenic medium from clinical samples, the first isolation of C. burnetii become observed using ACCM2 [99]. An affected person sample of the coronary heart valve inflamed with C. Burnetii endocarditis become incubated in 20 ml of ACCM2 and after 6-8 days of incubation, the increase was examined. ACCM2 produces numerous colonies at day 5 after incubation of an ACCM2 agar plate with a sample of the culture-positive [99]. Such specific possibilities could substantially promote the regular cultivation of C. Burnetii from scientific samples.

3.4 Pathology and Immunohistochemistry

The immune reaction produced in different organs by C. burnetii can be observed by pathological examination of tissue samples fixation and paraffin embedding. After hepatic biopsy specimen’s analysis, a typical fibrin-ring
granuloma with a “doughnut” feature can be detected during the primary infection [100]. In the bone marrow, these granulomas have also been identified. Pathological examination of vascular tissue and cardiac valves can also be descriptive during the persistent infection. Histological examination among the patients with C. burnetii endocarditis can disclose significant calcifications, fibrosis, slight inflammation and vascularization and less or no vegetation [101]. Such characteristics represent a passive “degenerative-like” infectious process. For the identification of C. burnetii in tissues, Immunohistochemical (IHC) detection is a more specific approach. An immunoperoxidase-based procedure along with a monoclonal antibody was used in this process. A procedure called “autoimmunochemistry” was developed by Lepidi et al. using antibodies from the patient’s serum. Samples from hepatic and valvular biopsy specimens and aortic grafts used the IHC procedure for the detection of C. burnetii [102].

3.5 Immuno-PCR

Immuno-PCR is a provocative process, incorporating the amplification potential of PCR with the precision and flexibility of ELISA, which permits enhancement insensitivity. This procedure has also been applied to different samples from infected individuals [103]. Immuno-PCR sensitivity is better as compared to ELISA and IFA (90% versus 35% and 25%, respectively) in sera assembled during the first two weeks after the emergence of symptoms [103]. Its specificity was assessed at 92%.

3.6 Skin Test

A skin check technique became suggested to look at the cellular feedback and to enhance the diagnosis of inflamed cows on the herd degree [104]. Intradermal injection of incredibly diluted inactivated vaccine (Coxevac, CEVA-Sant e Animale, Libourne, France) became applied for a skin test. Antigenic reactions induce with such diluted vaccine. A nodule of irregular size will develop on the site of injection if the animal has previously been infected by using q fever. Rural practitioners can without difficulty observe this test.

3.7 Prevention

In some situations, q fever is an occupational ailment. This has been verified by the primary rationalization of the sickness, which takes vicinity in a populace of slaughterhouse employees. In 1930, additionally, a member of a laboratory organization cultivating C. Burnetii turned into infected [105]. The primary type of individuals which infected with such ailment includes people receives uncovered to animals and laboratory workers who cultivating the bacterium. Instances that interact scientific workforce include folks that participate in autopsies of patients with q fever [105] or an obstetrician who regulated parturient ladies with q fever [106]. Instances among military officials have additionally been found.

3.8 Vaccination

Since 1989, Australia has the facility of vaccination (Q-Vax; CSL Biotherapies, Parkville, Victoria, Australia) [107]. It is licensed in Australia with whole-cell formalin-inactivated vaccine. A randomized control trial was performed among the 200 slaughterhouse workers to determine its efficacy. Seven cases were reported in the control group during the 15 months of follow up and not no case was reported in the vaccinated group [108]. However, this vaccine can provoke local reactions and before vaccination for Q fever patients should be assessed with a cutaneous test (Q-Vax skin test) to avoid severe side effects. This vaccine is recommended by the Australian Veterinary Association (AVA) for all veterinarians, veterinary nurses and veterinary students. A recent survey performed in Australia confirmed the vaccination of 74% of veterinarians and 29% of veterinary nurses [107]. In 2002, also a nationally sponsor vaccination program was started in the country [108]. Program fidelity was 100% among slaughterhouse workers and 43% among farmers. After this massive campaign, Q fever cases decreased by 50%, and the number of hospitalizations also decreased [108]. During the epidemic in the southeast of the Netherlands in 2011, a vaccination movement started targeted people at risk for advancement to endocarditis and vascular infections [109]. To date, no other country has started a massive vaccination program among occupationally exposed persons. Awareness campaigns about the risk for C. burnetii infection in these areas will be helpful in the reduction of infection and early diagnosis of the infection.

3.9 Isolation

All manipulations must be performed in a biosafety level 3 (BSL3) laboratory with
appropriate personal protective equipment (PPE) concerning the prevention of transmission of contamination to some of the laboratory workers dealing with C. burnetii cultures. For health care workers to convey out autopsies on patients remarkable to have died from q fever, have to be used N95 respiration protection masks is supported. Similar guidelines should be carried out for obstetrical personnel who are in exposure with parturient girls detected with C. burnetii infection. Furthermore, contaminated surfaces with C. burnetii must be disinfecting with a dual quaternary ammonium-detergent compound [110]. Family bleach dilution is likewise an effective solution for disinfection.

3.10 Perspective and Future Challenges

A more precise evaluation of the risk factors in the progression to C. burnetii endocarditis will require specific studies. The consideration of individual susceptibility factors will permit stronger directions through active treatment to prevent progression to this still-severe disease. By multiplex PCR techniques the systematic diagnosis of C. burnetii in syndromes such as hepatitis, fever, endocarditis and pneumonia during pregnancy should permit identification and treatment of more cases in the world. Finally, for endocarditis and vascular infections unique therapeutic techniques with routes and better sensitivity should be developed. New techniques such as unique antibiotic combinations, in vitro observation and randomized experiments, should be compared with the therapeutics methods that have been used for the last 20 years are compulsory and will be a major tool of future research.

4. CONCLUSION

The current review explained the molecular mechanism and characteristics of C. burnetii pathogenesis. Current approaches used for the diagnosis of disease, treatment and vaccine strategies were also described. Since the 1930s Q fever has been presented but not described as an emerging zoonosis. By the advancement of diagnostic approaches such as PCR and serological assays allow exact identification of the infected group or increased surveillance of professionals with flu-like symptoms or unexplained fever could be helped in the re-emergence of Q fever. To date still, Q fever remains poorly understood. Lack of awareness among the public is the one of major concerns related to the underdiagnosing and distribution of Q fever cases. The prevalence of Q fever can have a significant economic impact on animal trade, reproduction and the commercialization of different animal products. To prevent from burden of Q fever prevalence there should be introduced some novel diagnostic techniques and to call a massive campaign about the awareness of such disease.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

ACKNOWLEDGEMENT

All the authors sincerely thank Mr. Arslan Habib for his support in designing, formatting, and analyzing the data for the manuscript. Special thanks to all the authors for their technical support towards the manuscript review.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. The community summary report on trends and sources of zoonoses and zoonotic agents and food-borne outbreaks in the European Union in 2008. EFSA Journal 2010; 8(1): 241–247.
2. Cutler SJ, Bouzid M, Cutler RR. Q fever. J Infect. 2007;54(4):313–8.
3. Domingo P, Munoz C, Franquet T, Gurguí M, Sancho F, Vazquez G. Acute Q fever in adult patients: report on 63 sporadic cases in an urban area. Clin Infect Dis. 1999;29(4):874-9.
4. Center for Disease Control and prevention. Symptoms, Diagnosis, and Treatment of Q fever.
parasitophorous vacuole requires bacterial RpoS. Maturation of the Coxiella burnetii parasitophorous vacuole requires bacterial protein synthesis but not replication. Cell Microbiol. 2003;5(7):469-80.

Habib A, Razzak KS, Imran M, Khalid B. Elimination of Hepatitis C Virus: A Goal of WHO. Vigyan Varta. 2020;1:56-9.

Rehacek J, Tarasevich IV. Q fever. In: Acari-borne rickettsiae & rickettsioses in Eurasia. Veda Publishing House of the Slovak Academy of Sciences. Bratislava 1988.

Weisburg WG, Dobson ME, Samuel JE, Dasch GA, Mallavia LP, Baca O, Mandelco L, Sechrist JE, Weiss E, Woese CR. Phylogenetic diversity of the Rickettsiae. J bacteriol. 1989;171(8):4202-6.

Stein A, Saunders NA, Taylor AG, Raoul D. Phylogenetic homogeneity of Coxiella burnetii strains as determined by 16S ribosomal RNA sequencing. FEMS Microbiol Lett. 1993;113(3):339-44.

Baca OG, Paretsky D. Q fever and Coxiella burnetii: a model for host-parasite interactions. Microbiol Rev. 1983;47(2):127.

Bouvery NA, Souriau A, Lechopier P, Rodolakis A. Experimental Coxiella burnetii infection in pregnant goats: excretion routes. Vet Res. 1983;47(2):127.

Samuel JE, Kiss K, Varghees S. Molecular pathogenesis of Coxiella burnetii in a genomics era. Ann N Y Acad Sci. 2003;(1):653-63.

Swanson MS, Fernandez-Moreia E. A microbial strategy to multiply in macrophages: the pregnant pause, Traffic 3 (2002) 170–177.

Seshadri R, Samuel JE. Characterization of a stress-induced alternate sigma factor, RpoS, of Coxiella burnetii and its expression during the development cycle. Infect Immun. 2001;69(8):4874–83.

Heinzen RA, Hackstadt T, Samuel JE. Developmental biology of Coxiella burnetii. Trends Microbiol. 1999;7(4):149-54.

Melníčáková J, Lukáčová M, Howe D, Heinzen RA, Barák I. Identification of Coxiella burnetii RpoS-dependent Promoters. Ann N Y Acad Sci. 2003;990(1):591-5.

Howe D, Mallavia LP. Coxiella burnetii exhibits morphological change and delays phagolysosomal fusion after internalization by J774A. 1 cells. Infect Immun. 2000;68(7):3815-21.

Howe D, Melníčáková J, Barák I, Heinzen RA. Maturation of the Coxiella burnetii parasitophorous vacuole requires bacterial protein synthesis but not replication. Cell Microbiol. 2003;5(7):469-80.

Habib A, Principe UE, Ashraf Z, Imran M. Epidemiology of Rabies and the Control Challenges. Biotic Res Today. 2020;2(11):1201-4.

Seshadri R, Paulsen IT, Eisen JA, Read TD, Nelson KE, Nelson WC, Ward NL, Tettelin H, Davidsen TM, Beanan MJ, Deboy RT. Complete genome sequence of the Q-fever pathogen Coxiella burnetii. Proc Natl Acad Sci. 2003;100(9):5455-60.

Zamboni DS, McGrath S, Rabinovitch M, Roy CR. Coxiella burnetii express type IV secretion system proteins that function similarly to components of the Legionella pneumophila Dot/Icm system. Mol Microbiol. 2003;49(4):965-76.

Zusman T, Yerushalmi G, Segal G. Functional similarities between the icm/dot pathogenesis systems of Coxiella burnetii and Legionella pneumophila. Infect Immun. 2003;71(7):3714-23.

Dellassacasagrande J, Ghigo E, Machergui-El S, Hammami, Toman R, Raoul D, Capo C, Mege JL, alpha(y) beta (3) integrin and bac- terial lipopolysaccharide are involved in Coxiella burnetii-stimulated production of tumor necrosis factor by human monocytes. Infect Immun. 2000;68:5673-5678.

Honstettre A, Ghigo E, Moynault A, Capo C, Toman R, Akira S, Takeuchi O, Lepidi H, Raoul D, Mege JL. Lipopolysaccharide from Coxiella burnetii is involved in bacterial phagocytosis, filamentous actin reorganization, and inflammatory responses through Toll-like receptor 4. J Immunol. 2004;172(6):3695-703.

Janssens S, Beyaert R. Role of Toll-like receptors in pathogen recognition. Clin Microbiol Rev. 2003;16(4):637-46.

Vasselon T, Detmers PA. Toll receptors: a central element in innate immune responses. Infect Immun. 2002;70(3):1033-41.

Marrie TJ, Durant H, Yates L. Community-acquired pneumonia requiring hospitalization: 5-year prospective study. Rev Infect Dis. 1989;11(4):566-99.

Liebman D, Lieberman D, Boldur I, Manor E, Hoffman S, Schlaeffer F, Porath A. Q-fever pneumonia in the Negev region of Israel: a review of 20 patients hospitalised over a period of one year. J Infect. 1995;30(2):135-40.
28. Dupont HT, Thiron XA, Raoult D. Q fever serology: cutoff determination for microimmunofluorescence. Clin Diagn Lab Immunol. 1994;1(2):189.

29. Rice PS, Kudesia G, McKendrick MW, Cullen DR. Coxiella burnetii serology in granulomatous hepatitis. J Infect. 1993;27(1):63-6.

30. Honarmand H. Q Fever: an old but still a poorly understood disease. Interdiscip Perspect Infect Dis. 2012 Nov 19;2012.

31. Maurin M, Raoult DF. Q fever. Clin Microbiol Rev. 1999;12(4):518-53.

32. Tseleltis Y, Gikas A, Kofferidis D, Kyriakakis E, Lydatski N, Bouros D, Tsaparas N. Q fever in the Greek Island of Crete: epidemiologic, clinical, and therapeutic data from 98 cases. Clin Infect Dis. 1995;20(5):1311-6.

33. Santoro D, Giura R, Colombo MC, Antonelli P, Gramegna M, Gandola O, Gridavilla G. Q fever in Como, northern Italy. Emerg Infect Dis. 2004;10(1):159.

34. Takahashi H, Tokue Y, Kikuchi T, Kobayashi T, Gomi K, Goto I, Shiraiishi H, Fukushima H, Hirai K, Nukiwa T, Watanabe A. Prevalence of community-acquired respiratory tract infections associated with Q fever in Japan. Diagn Microbiol Infect Dis. 2004;48(4):247-52.

35. Woodtayakorn J, 4. Dupuis G, Petite J, Peter O, Vouilloz M. An important outbreak of human Q fever in a Swiss Alpine valley. Int J Epidemiol 1987;16:282–7. 5.

36. Habib A, Anjum KM, Ashraf Z, Principe UE, Hussain Z, Khalid B, Ramzan M. Global Epidemiology of COVID-19 and the Risk of Second Wave. J Drug Deliv Ther. 2020;11(1):1-2.

37. Sampere M, Font B, Font J, Sanfeliu I, Segura F. Q fever in adults: review of 66 clinical cases. Eur J Clin Microbiol Infect Dis. 2003;22(2):108-10.

38. Sobradillo V, Ansola P, Baranda F, Corral C. Q fever pneumonia: a review of 164 community-acquired cases in the Basque country. Eur Respir J. 1989;2(3):263-6.

39. Chong AK, Brooy JL, Norton R, Masson J. Q fever: a recent outbreak in Townsville. Intern Med J. 2003;33(4):208-10.

40. Kocianová E, Kovacova EI, Literák I. Comparison of virulence of Coxiella burnetii isolates from bovine milk and from ticks. Folia parasitol. 2001;48(3):235-9.

41. Masala G, Porcu R, Sanna G, Chessa G, Cillara G, Chisu V, Tola S. Occurrence, distribution, and role in abortion of Coxiella burnetii in sheep and goats in Sardinia, Italy. Vet Microbiol. 2004;99(3-4):301-5.

42. Komiyi T, Toriniwa H, Sadamasu K, Fukushima H, Hirai K, Arashima Y, Kato K, Arakawa Y. Epidemiological survey on the route of Coxiella burnetii infection in an animal hospital. J Infect Chemother. 2003;9(2):151-5.

43. Thiele D, Willems H, Haas M, Krauss H. Analysis of the entire nucleotide sequence of the cryptic plasmid QpH1 from Coxiella burnetii. Eur J Epidemiol. 1994;10(4):413-20.

44. Beare PA, Unsworth N, Andoh M, Voth DE, Omsland A, Gilk SD, Williams KP, Sobral BW, Kupco JJ, Porcella SF, Samuel JE. Comparative genomics reveal extensive transposon-mediated genomic plasticity and diversity among potential effector proteins within the genus Coxiella. Infect Immun. 2009;77(2):642-56.

45. Samuel JE, Frazier ME, Mallavia LP. Correlation of plasmid type and disease caused by Coxiella burnetii. Infect Immun. 1985;49(3):775-9.

46. Svraka S, Toman R, Skultety L, Slaba K, Homan WL. Establishment of a genotyping scheme for Coxiella burnetii. FEMS Microbiol Lett. 2006;254(2):268-74.

47. Glazunova O, Roux V, Freylikman O, Sekyeya Z, Fournous G, Tyczka J, Tokarevich N, Kovacova E, Marrie TJ, Raoul D. Coxiella burnetii genotyping. Emerg Infect Dis. 2005;11(8):1211.

48. Beare PA, Samuel JE, Howe D, Virtaneva K, Porcella SF, Heinzen RA. Genetic diversity of the Q fever agent, Coxiella burnetii, assessed by microarray-based whole-genome comparisons. J Bacteriol. 2006;188(7):2309-24.

49. Andoh M, Nagaoka H, Yamaguchi T, Fukushima H, Hirai K. Comparison of Japanese isolates of Coxiella burnetii by PCR-RFLP and sequence analysis. Microbiol Immunol. 2004;48(12):971-5.

50. Sekyeya Z, Roux V, Raoul D. Intraspecies diversity of Coxiella burnetii as revealed by com1 and mucZ sequence comparison. FEMS Microbiol Lett. 1999;180(1):61-7.

51. RJ H, GA H, EB R. Action of streptomycin in experimental infection with Q fever. Public health reports (Washington, DC: 1896). 1948;63(12):357-62.

52. Jackson EB. Comparative Efficacy of Several Antibiotics on Experimental Rickettsial Infections in Embryo nated
Eggs. Antimicrob Agents Chemother. 1951;1(4):231-41.

53. Yeaman MR, Mitscher LA, Baca OG. In vitro susceptibility of Coxiella burnetii to antibiotics, including several quinolones. Antimicrob Agents Chemother. 1987;31(7):1079-84.

54. Gikas A, Spyridaki I, Scoulica E, Psaroulaki A, Tselenitis Y. In vitro susceptibility of Coxiella burnetii to linezolid in comparison with its susceptibilities to quinolones, doxycycline, and clarithromycin. Antimicrob Agents Chemother. 2001;45(11):3276-8.

55. Brennan RE, Samuel JE. Evaluation of Coxiella burnetii antibiotic susceptibilities by real-time PCR assay. J Clin Microbiol. 2003;41(5):1869.

56. Boulos A, Rolain JM, Maurin M, Raoult D. Measurement of the antibiotic susceptibility of Coxiella burnetii using real time PCR. Int J Antimicrob Agents. 2004;23(2):169-74.

57. B Unsworth N, M Dawson R, D Wade J, Liu CQ. Susceptibility of intracellular Coxiella burnetii to antimicrobial peptides in mouse fibroblast cells. Protein Pep Lett. 2014;21(2):115-23.

58. Eldin C, Perreal C, Mahamat A, Djossou F, Edouard S, Raoult D. Antibiotic susceptibility determination for six strains of Coxiella burnetii MST 17 from Cayenne, French Guiana. Int Antimicrob Agents. 2015;46(5):600-2.

59. Rolain JM, Boulos A, Mallet MN, Raoult D. Correlation between ratio of serum doxycycline concentration to MIC and rapid decline of antibody levels during treatment of Q fever endocarditis. Antimicrob Agents Chemother. 2005;49(7):2673-6.

60. Rouli L, Rolain JM, El Filali A, Robert C, Raoult D. Genome sequence of Coxiella burnetii 109, a doxycycline-resistant clinical isolate.

61. ROLAIN JM, Lambert F, Raoult D. Activity of telithromycin against thirteen new isolates of C. burnetii including three resistant to doxycycline. Ann N Y Acad Sci. 2005;1063(1):252-6.

62. Jabarit-Aldighieri N, Torres H, Raoult D. Susceptibility of Rickettsia conorii, R. rickettsii, and Coxiella burnetii to PD 127,391, PD 131,628, pefloxacin, ofloxacin, and ciprofloxacin. Antimicrob Agents Chemother. 1992;36(11):2529-32.

63. Raoult D. Treatment of Q fever. Antimicrob Agents Chemother. 1993;37(9):1733.

64. Drancourt M, Gallais H, Raoult D, Estrangin E, Mallet MN, De Micco P. Ofloxacin penetration into cerebrospinal fluid. J Antimicrob Chemother. 1998;22(2):263-5.

65. Musso D, Drancourt M, Osscini S, Raoult D. Sequence of quinolone resistance-determining region of gyrA gene for clinical isolates and for an in vitro-selected quinolone-resistant strain of Coxiella burnetii. Antimicrob Agents Chemother. 1996;40(4):870-3.

66. Spyridaki I, Psaroulaki A, Aransay A, Scoulica E, Tselenitis Y. Diagnosis of quinolone-resistant Coxiella burnetii strains by PCR-RFLP. J Clin Lab Anal. 2000;14(2):59-63.

67. Enright JB, Sadler WW, Thomas RC. Pasteurization of milk containing the organism of Q fever. Am J Public Health. 1957;47(6):695-700.

68. Raoult D, Torres H, Drancourt M. Shell-vial assay: evaluation of a new technique for determining antibiotic susceptibility, tested in 13 isolates of Coxiella burnetii. Antimicrob Agents Chemother. 1991;35(10):2070-7.

69. Sandoz KM, Sturdevant DE, Hansen B, Heinzen RA. Developmental transitions of Coxiella burnetii grown in axenic media. J Microbiol Methods. 2014;104:104-10.

70. RJ H, GA H, EB R. Action of streptomycin in experimental infection with Q fever. Public health reports (Washington, DC: 1896). 1948;63(12):357-62.

71. B Unsworth N, M Dawson R, D Wade J, Liu CQ. Susceptibility of intracellular Coxiella burnetii to antimicrobial peptides in mouse fibroblast cells. Protein Pep Lett. 2014;21(2):115-23.

72. Minnick MF, Hicks LD, Battisti JM, Raghaven R. Pentamidine inhibits Coxiella burnetii growth and 23S rRNA intron splicing in vitro. J Antimicrob Agents. 2010;36(4):380-2.

73. Botelho-Nevers E, Singh S, Chiche L, Raoult D. Effect of omeprazole on vacuole size in Coxiella burnetii-infected cells. J Infect. 2013;66(3):288-9.

74. Maurin M, Benoïel AM, Bongrand P, Raoult D. Phagolysosomes of Coxiella burnetii-infected cell lines maintain an acidic pH during persistent infection. Infect Immun. 1992;60(12):5013-6.

75. Raoult D, Drancourt M, Vestris G. Bactericidal effect of doxycycline associated with lysosomotropic agents on
Differentiation of Coxiella burnetii Strains

76. Raoult D, Houpiakian P, Dupont HT, Riss JM, Arditi-Djiane J, Brouqui P. Treatment of Q fever endocarditis: comparison of 2 regimens containing doxycycline and ofloxacin or hydroxychloroquine. Archi Intern Med. 1999;159(2):167-73.

77. Cisak E, Chmielewska-Badora J, Mackiewicz B, Dutkiewicz J. Prevalence of antibodies to Coxiella burnetii among farming population in eastern Poland. Ann Agric Environ Med. 2003;10(2):265-7.

78. Waag DM, England MJ, Pitt MLM. Comparative efficacy of a Coxiella burnetii chloroform: methanol residue (CMR) vaccine and a licensed cellular vaccine (Q-Vax) in rodents challenged by aerosol. Vaccine 1997;15(16):1779-1783.

79. Waag DM. Coxiella burnetii: host and bacterial responses to infection. Vaccine. 2007;25(42):7288-95.

80. Knap J. The clinic of Q fever in humans. Anusz Z. Q fever in humans and animals, ART Olsztyn. 1995:85-109.

81. Annual epidemiological report on communicable diseases in Europe;2010. Available:www.ecdc.europa.eu/en/publications/publications/1011_sur_annual_epidemiological_report_on_communicable_diseases_in_europe (Access 2011.07.22).

82. Fournier PE, Marrie TJ, Raoult D. Diagnosis of Q fever. J Clin Microbiol. 1998;36(7):1823-34.

83. Peter O, Dupuis G, Bee D, Lüthy R, Nicolet J, Burgdorfer W. Enzyme-linked immunosorbent assay for diagnosis of chronic Q fever. J Clin Microbiol. 1988;26(10):1978-82.

84. Cowley R, Fernandez F, Freemantle W, Rutter D. Enzyme immunoassay for Q fever: comparison with complement fixation and immunofluorescence tests and dot immuno blotting. J Clin Microbiol. 1992;30(9):2451-5.

85. Marrie TJ. Coxiella burnetii pneumonia. Eur Respir J. 2003;21(4):713-9.

86. Mikołajczyk E, Lewińska Z, Łojewska R, Rumin W, Kruszewska D. Serologic reactions in humans during the outbreak of Q fever. Przegl Epidemiol. 1986;40(4):342-8.

87. Mallavía LP, Whiting LL, Minnick MF, Heinzén R, Foreman M, Baca OG, Frazier ME. Strategy for Detection and Differentiation of Coxiella burnetii Strains Using the Polymerase Chain Reaction. Ann N Y Acad Sci. 1990;590(1):572-81.

88. Ibrahim A, Norlander L, Macellaro A, Sjöstedt A. Specific detection of Coxiella burnetii through partial amplification of 23S rDNA. Eur J Epidemiol. 1997;13(3):329-34.

89. Fenollar F, Raoult D. Molecular genetic methods for the diagnosis of fastidious microorganisms. Apmis. 2004;112(11-12):785-807.

90. van Vliet S, Hol FJ, Weenink T, Galajda P, Keymer JE. The effects of chemical interactions and culture history on the colonization of structured habitats by competing bacterial populations. BMC Microbiol. 2014;14(1):1-6.

91. Rolain JM, Raoult D, Marmion BP, Harris RJ, Storm P, Ayres JG. Molecular detection of Coxiella burnetii in blood and sera during Q fever. Qjm. 2005;98(8):615-20.

92. Schneeberger PM, Hermans MH, van Hannen EJ, Schellekens JJ, Leenders AC, Wever PC. Real-time PCR with serum samples is indispensable for early diagnosis of acute Q fever. Clin Vaccine Immunol. 2010;17(2):286-90.

93. Wielders CC, Wijnbergen PC, Renders NH, Schellekens JJ, Schneeberger PM, Wever PC, Hermans MH. High Coxiella burnetii DNA load in serum during acute Q fever is associated with progression to a serologic profile indicative of chronic Q fever. J Clin Microbiol. 2013;51(10):3192-8.

94. Tilburg JJ, Melchers WJ, Pettersson AM, Rossen JW, Hermans MH, van Hannen EJ, Nabuurs-Franssen MH, de Vries MC, Horrevorts AM, Klaassen CH. Interlaboratory evaluation of different extraction and real-time PCR methods for detection of Coxiella burnetii DNA in serum. J Clin Microbiol. 2010;48(11):3923-7.

95. Kokcha S, Bittar F, Reynaud-Gaubert M, Mely L, Gomez C, Gaubert JY, Thomas P, Rolain JM. Pandoraea pulmonica chronic colonization in a cystic fibrosis patient, France. New Microbes New Infect. 2013;1(2):27-9.

96. Eldin C, Angelakis E, Renvoisé A, Raoult D. Coxiella burnetii DNA, but not viable bacteria, in dairy products in France. AmJ Trop Med Hyg. 2013;88(4):765-9.

97. Gouriet F, Fenollar F, Patrice JY, Drancourt M, Raoult D. Use of shell-vial cell culture assay for isolation of bacteria.
from clinical specimens: 13 years of experience. J Clin Microbiol. 2005;43(10):4993-5002.

98. Wang J, Mendelsohn R, Dinar A, Huang J, Rozelle S, Zhang L. The impact of climate change on China's agriculture. Agri Econ. 2009;40(3):323-37.

99. Boden K, Wolf K, Hermann B, Frangoulidis D. First isolation of Coxiella burnetii from clinical material by cell-free medium (ACCM2). Eur J Clin Microbiol Infect Dis. 2015;34(5):1017-22.

100. Lee M, Jang JJ, Kim YS, Lee SO, Choi SH, Kim SH, Yu E. Clinico-pathologic features of Q fever patients with acute hepatitis. Korean J Pathol. 2012;46(1):10.

101. Argov O, Weintraub M, Charach G. "Doughnut" granulomas from erythema nodosum in acute Q fever. Isr Med Assoc J. 2008;10(3):241-2.

102. Lepidi H, Fournier PE, Karcher H, Schneider T, Raoult D. Immunohistochemical detection of Coxiella burnetii in an aortic graft. Clin Microbiol Infect. 2009;15:171-2.

103. Malou N, Renvoise A, Nappez C, Raoult D. Immuno-PCR for the early serological diagnosis of acute infectious diseases: the Q fever paradigm. Eur J Clin Microbiol Infect Dis. 2012;31(8):1951-60.

104. Guatette R, Joly A, Rodolakis A, Cochonneau D, Sarradin P, Seegers H, Remdy D, Beaudeau F. Prévention de l'excrétion de Coxiella burnetii l'aide d'un vaccin dit phase I (Coxevac® en troupeaux bovines laitiers infectés). Rencontres autour des Recherches sur les Ruminants. 2008;15:59-62.

105. Davis GE, Cox HR, Parker RR, Dyer RE. A filter-passing infectious agent isolated from ticks. Public Health Rep. 1938;53(52):2259-311.

106. Gerth HJ, Leidig U, Riemenschneider T. Q-fieber-epidemie in einem Institut für Humanpathologie. Dtsch Med Wochenschr. 1982;107(37):1391-5.

107. Raoult D, Stein A. Q fever during pregnancy--a risk for women, fetuses, and obstetricians. N Engl J Med. 1994;330(5):371-.

108. Wilson G, Bryan J, Cranston K, Kitzes J, Nederbragt L, Teal TK. Good enough practices in scientific computing. PLoS Comput Biol. 2017;13(6):1005510.

109. Shapiro RA, Siskind V, Schofield FD, Stallman N, Worswick DA, Marmion BP. A randomized, controlled, double-blind, cross-over, clinical trial of Q fever vaccine in selected Queensland abattoirs. Epidemiol Infect. 1990;104(2):267-73.

110. European Centre for Disease Prevention and Control. Introduction to the Annual Epidemiological Report Stockholm: ECDC; 2019. Available: https://ecdc.europa.eu/en/annual-epidemiological-reports/methods

111. Arricau-Bouvy N, Rodolakis A. Is Q fever an emerging or re-emerging zoonosis. Vet Res. 2005;36(3):327-49.

112. Schelling E, Diguimbaye C, Daoud S, Nicolet J, Boerlin P, Tanner M, Zinsstag J. Brucellosis and Q-fever seroprevalences of nomadic pastoralists and their livestock in Chad. Prevent Vet Med. 2003;61(4):279-93.

113. Masala G, Porcu R, Sanna G, Chessa G, Cillara G, Chisu V, Tola S. Occurrence, distribution, and role in abortion of Coxiella burnetii in sheep and goats in Sardinia, Italy. Vet Microbiol. 2004;99(3-4):301-5.

114. Capuano F, Landolfi MC, Monetti DM. Influence of three types of farm management on the seroprevalence of Q fever as assessed by an indirect immunofluorescence assay. Vet Rec. 2001;149(22):669-71.

115. Cetinkaya B, Kalender H, Ertas HB, Muz A, Arslan N, Onorg H, Gurcay M. Seroprevalence of coxiellosis in cattle, sheep and people in the east of Turkey. Vet Rec. 2000;146(5):131-6.

116. Ibrahim IN, Okabayashi T, Lestari EW, Yanase T, Muramatsu Y, Ueno H, Morita C. Serosurvey of wild rodents for rickettsioses (spotted fever, murine typhus and Q fever) in Java Island, Indonesia. Eur J Epidemiol. 1999;15(1):89-93.

117. Komiy T, Sadamasu K, Kang MI, Tsuboshima S, Fukushima H, Hirai K. Seroprevalence of Coxiella burnetii infections among cats in different living environments. J Vet Med Sci. 2003;56(9):1047-8.

118. Boschini A, Di Perri G, Legnani D, Fabbri P, Ballarini P, Zuconni R, Boros S, Rezza G. Consecutive epidemics of Q fever in a residential facility for drug abusers: impact on persons with human immunodeficiency virus infection. Clin Infect Dis. 1999;28(4):866-72.

119. Tissot-Dupont H, Torres S, Nezri M, Raoult D. Hyperendemic focus of Q fever related to sheep and wind. Am J Epidemiol. 1999;150(1):67-74.
120. Potasman I, Rzotkiewicz S, Pick N, Keysary A. Outbreak of Q fever following a safari trip. Clin Infect Dis. 2000;30(1):214-5.
121. Reintjes R, Hellenbrand W, Dusterhaus A. Outbreak of Q-fever in Dortmund in the summer of 1999-Results of an epidemiological outbreak study. Gesundheitswesen. 2000;62(11):609-14.
122. Arricau-Bouvery N, Rodolakis A. Is Q fever an emerging or re-emerging zoonosis. Vet Res. 2005;36(3):327-49.
123. Hatchette TF, Hudson RC, Schlech WF, Campbell NA, Hatchette JE, Ratnam S, Raoult D, Donovan C, Marrie TJ. Goat-associated Q fever: a new disease in Newfoundland. Emerg Infect Dis. 2001;7(3):413.
124. Gardon J, Héraud JM, Laventure S, Ladam A, Capot P, Fouquet E, Favre J, Weber S, Hommel D, Hulin A, Couratte Y. Suburban transmission of Q fever in French Guiana: evidence of a wild reservoir. J Infect Dis. 2001;184(3):278-84.
125. Gilroy N, Formica N, Beers M, Egan A, Conaty S, Marmion B. Abattoir-associated Q fever: a Q fever outbreak during a Q fever vaccination program. Aust N Z J Public Health. 2001;25(4):362-7.
126. Steiner H, Raveh D, Rudensky B, Paz E, Jerassi Z, Schlesinger Y, Yinnon A. Outbreak of Q fever among kitchen employees in an urban hospital. Eur J Clin Microbiol Infect Dis. 2001;20(12):898.
127. Kawamoto T, Ogawa M, Kishimoto T, Uchida Y, Kato K, Kawamoto A, Yamashita T. Three imported cases of acute Q fever following an inspection tour to Australia and New Zealand. Kansenshogaku zasshi. Jpn Assoc Infect Dis. 2002;76(12):1030-4.
128. Carrieri M, Tissot-Dupont H, Rey D, Brousse P, Renard H, Obadia Y, Raoult D. Investigation of a slaughterhouse-related outbreak of Q fever in the French Alps. Eur J Clin Microbiol Infect Dis. 2002;21(1):17-21.
129. Komiy A, Toriniwa H, Sadamasu K, Fukushi H, Hirai K, Arashima Y, Kato K, Arakawa Y. Epidemiological survey on the route of Coxiella burnetii infection in an animal hospital. J Infect Chemother. 2003;9(2):151-5.
130. Splino M, Beran J, Chlibek R. Q fever outbreak during the Czech Army deployment in Bosnia. Military Med. 2003;168(10):840-2.
131. Chong AK, Brooy JL, Norton R, Masson J. Q fever: a recent outbreak in Townsville. Intern Med J. 2003;33(4):208-10.
132. Berri M, Rousset E, Champion JL, Arricau-Bouvery N, Russo P, Pepin M, Rodolakis A. Ovine manure used as garden fertiliser as a suspected source of human Q fever. Vet Rec. 2003;153(9):269-70.
133. Santoro D, Giura R, Colombo MC, Antonelli P, Gramenga M, Candola O, Gridavilla G. Q fever in Como, northern Italy. Emerg Infect Dis. 2004;10(1):159.
134. Beare PA, Unsworth N, Andoh M, Voth DE, Omsland A, Gilk SD, Williams KP, Sobral BW, Kupko III JJ, Porcella SF, Samuel JE. Comparative genomics reveal extensive transposon-mediated genomic plasticity and diversity among potential effector proteins within the genus Coxiella. Infect Immun. 2009;77(2):642-56.

© 2021 Habib et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/70278